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CONTENTS

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE CRANIAL MORPHOLOGY OF SOME RANID GENERA OF FROGS.—Part II.

BY L. S. RAMASWAMI,

Department of Zoology, Central College, Bangalore.

Introduction.

IN my previous paper (author, 1932) I described some aspects of the cranial morphology of some species of the two genera *Rhacophorus* and *Philautus*. It was pointed out that the examined species of *Philautus* could not be merged with *Rhacophorus* though some systematists like Smith maintain that a large number of species of *Philautus* are degenerate forms of *Rhacophorus*, or they are individuals that have been arrested in their development. The present communication is a contribution to our knowledge of the cranial morphology of three species of the genus *Rana*, commonly met with in South India. The object of this study is to place on record certain cranial character-istics of the South Indian Ranid forms and to compare them with those of the European forms like *Rana fusca* and *Rana esculenta* and also with the South African form, *Rana grayi*.

I have selected three thoroughly aquatic forms for study, *viz.*, *Rana hexadactyla* Lesson, *Rana cyanophlyctis* Schneid, and *Rana curtipes* Jerdon. According to Boulenger (1890) the first reference to *R. hexadactyla* is made in *Belang. Voy. Ind. Or. Zool.*, p. 331 by Lesson, to *R. cyanophlyctis* in *Hist. Amph.* i., p. 137 and to *R. curtipes* in *Journ. As. Soc. Bengal,* **22**, p. 532, 1858. I am unable to comment upon the nature of description given about these forms, since I was not able to secure these papers for reference.

The earliest work on the development and morphology of the Batrachian skull is by Parker (1881). He records in this paper the cranial morphology of some Indian Ranid forms like *R. Kuhli, R. hexadactyla, R. gracilis, R. cyanophlyctis, R. pygmœa* and *R. tigrina.* Gaupp (1896–1904), on the other hand, describes the anatomy of the entire head of European forms like *R. fusca* and *R. esculenta* and his work is of classic importance though some points in his work require verification.

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Material and Method.

The adult Ranid specimens were all collected alive and fixed in Bouin's fluid. The heads were decalcified in 70% alcohol containing 3% nitric acid. Sections 12 microns thick were cut and stained in Hæmalum-eosin and Hæmalum-picroindigocarmine.

The Olfactory Region.

The cavities of the narial region of the three species of *Rana* are similar to the ones described for *Rana fusca* by Gaupp. In the anterior region of the frogs studied by me, the sections show the presence of both the prenasal cartilages,—cartilago prenasalis superior and cartilago prenasalis inferior. The premaxilla invests both these cartilages. The cartilago prenasalis superior depends from the cartilago obliqua while the inferior cartilage depends from the solum.

The plica, in all the Ranid forms described so far, is reported to depend from the tectum nasi but in forms like the South Indian engystomatidæ (*Microhylidæ* of Parker, 1934) (author, 1932), *Glyphoglossus* (author, 1932 a), *Rhacophorus* and *Philautus* (author, 1934), *Rana grayi* (du Toit, 1933), *Phrynomerus* (de Villiers, 1930), *Cacosternum* (de Villiers, 1931), *Hemisus* (de Villiers, 1931 a) and *Bufo* (Schoonees, 1930), it depends from the cartilago obliqua. Judging by the weight of evidence in support of the obliquous suspension of the plica it may be generalised that in Anura, the plica invariably depends from the cartilago obliqua and not from the tectum proper.

The recessus sacciformis,—"a cavity which, on the one hand, communicates with the vestibulum, and on the other hand, with the infundibulum and the cavum medium where these two latter cavities communicate with each other" is absent from the three species of *Rana* studied. The presence of a recessus sacciformis is prominently noticed in the European species of *Rana*, in which the disposition of the organ conforms with the description given above.

The disposition of the laminal cartilages is normal and in close association with the superior laminal cartilage, the septomaxillary bone makes its appearance. The septomaxillary is usually considered as a membrane bone appearing as an investment of the superior laminal cartilage. The bone is separated from the cartilage by the intervening connective tissue. This view is accepted by Goodrich (1930), de Villiers and all modern anatomists. But Lapage (1928), working on the septomaxillary of Urodela and Anura reports that the bone is definitely cartilaginous in origin. This view, though not accepted by all modern anatomists, is not altogether obsolete. In forms like *Kaloula pulchra*, Gray (*K. pulchra taprobanica*, Parker, 1934), the bone

makes its appearance as an ossification in the superior laminal cartilage thereby supporting the observations of Lapage. William K. Parker (1881) in his monograph on the structure and development of the skull in Batrachia (Part III) refers to the occurrence or otherwise of this bone in a large number of Anuran forms. He describes that in the specimen of *Rana hexadactyla*
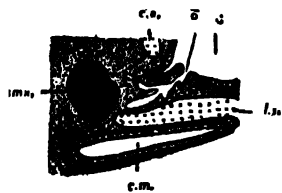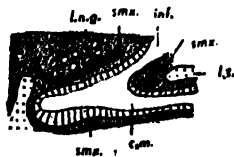
Fig. 1A.   Fig. 1B.   Fig. 1C.

Trans-sections in the region of the septomaxillary bone of *R. hexadactyla*.

an. ty.	..	Annulus tympanicus.	*op.R.*	.. Opening of the Rachendrüse.
b.c.	..	Buccal epithelium.		
b.v.	..	Blood vessel.	*ot.p.*	.. Processus oticus.
c.a.	..	Cartilago alaris.	*ot.c.*	.. Otic portion of the
ch.	..	Choana.		transitional cartilage.
c.m.	..	Cavum medium.	*parq.*	.. Paraquadrate.
c.par.	..	Crista parotica.	*pars.*	.. Parasphenoid.
c.p.ot.	..	Cartilaginous projection from the otic capsule.	*pal.*	.. Palatine.
			p.a.p.	.. Pars ascendens plectri.
c.pr.	..	Cavum principale.	*p.bas.*	.. Processus basalis.
c.s.	..	Cartilaginous support for the eminentia.	*p.e.p.*	.. Pars externa plectri.
			p.m.p.	.. Pars media plectri.
c.tr.c.	..	Cristal portion of the transitional cartilage.	*p.ptg.*	.. Processus pterygoideus.
			p.q.	.. Processus quadratus.
d.n.l.	..	Ductus nasolacrimalis.	*pr.f.*	.. Foramen prooticum.
e.t.	..	Eustachian tube.	*prot.*	.. Prootic bone.
f.p.	..	Frontoparietals.	*p.t.*	.. Planum terminale.
gl.	..	Glands surrounding the cavum principale.	*ptg.*	.. Pterygoid bone.
			ptg.b.	.. Processus basalis invaded by the pterygoid bone.
inf.	..	Infundibulum.		
l.n.g.	..	Lateral nasal glands.	*Q.*	.. Quadrate cartilage.
l.s.	..	Lamina superior.	*Qm.*	.. Quadratomaxillary.
max.	..	Maxilla.	*R.*	.. Rachendrüse.
Md.	..	Mundwinkeldrüse.	*s.e.c.*	.. Subethmoidal cartilage.
me.	..	Middle ear.	*sk.*	.. Skin.
mus.	..	Muscle.	*smx.*	.. Septomaxillary.
nas.	..	Os nasale.	*sph.*	.. Sphenethmoid.
n.s.	..	Septum nasale.	*t.n.*	.. Tectum nasi.
ne.	..	Nerve.	*t.p.*	.. Tonsillar patch.
o.c.	..	Otic capsule.	*tr.c.*	.. Transitional cartilage.
op.	..	Operculum.	*v.*	.. Vomer.
op.gl.	..	Opening of the glands surrounding the cavum principale into it.	*w.p.*	.. Worm parasite.
			V.ne.	.. Branch of the trigeminal nerve.

examined by him there is "a small septomaxillary on the right side only, but the nasal angle in its ascent has a solid bony mass formed in it." In the other Indian and extrapeninsular species examined by him, *viz.*, *Rana Kuhli*, *Rana gracilis*, *Rana pygmœa* and *Rana tigrina* the absence of septo-maxillary is noted, while in *Rana cyanophlyctis* "a small sigmoid septo maxillary" is reported by him. The same author also describes the occur-rence of ossifications in the laminal cartilages of other examples, as the septomaxillary. Having examined the sections of *R. hexadactyla*, *R. cyano-phlyctis*, *R. curtipes* and also that of *R. tigrina*, I have come to the conclu-sion that the observations of Parker (1881) with regard to *R. hexadactyla* and *R. tigrina* are not borne out by my studies. In my sections no lopsided development of the septomaxillary is noticed in *R. hexadactyla*, nor is the complete absence of the investing bone observed in *R. tigrina*. In *Rana hexadactyla* the bone appears as a small oval piece (Fig. 1A, *smx.*) above the lamina superius (*l.s.*) more towards the epidermal side of the cavum medium (*c.m.*) Posteriorly, the bone becomes hollowed out and one end of it invests the lamina superius while the other end bends over the infundibular (*inf.*) part of the cavum principale. Slightly posterior to the region where the infundibulum opens into cavum medium the septomaxillary is trifid (1B, *smx.*); one investment is noticed on the lamina superius while the other is seen on the lamina inferius and the third is situated externally to the infun-dibulum and internally to the glandulæ nasalis lateralis (*l.n.g.*). The investment noticed on the lamina superius disappears first; while the others unite to form a single piece at the region the cavum medium disengages a part from it as the nasolacrimal duct (*d.n.l.*). The bony piece referred to above also disappears from sections at the region where the planum terminale is fully formed. This description of the septomaxillary corresponds to that given for the South African form *Rana gray* by du Toit (1933). The dis-position of the bone in the other two species of *Rana* is almost similar to the one described for *R. hexadactyla*. In *R. cyanophlyctis* the bone appears as a single piece on the dorsal aspect of the lamina inferius. Posteriorly two more limbs make their appearance; a large one is added internally to the first and a smaller one is noticed under the lamina superius. The two external ones unite to form a forked single piece and the superior laminal investment remains the same (Fig. 2A, *smx.*); in the region of the infundibulum the forked external piece united with the internal one to form a single invest-ment. Fig. 2B is drawn to show the disposition of the bony pieces in the region where the infundibulum gains access into the cavum medium. After the formation of the ductus nasolacrimalis, the investment on the lamina superius disappears, while a small portion of the septomaxillary persists in
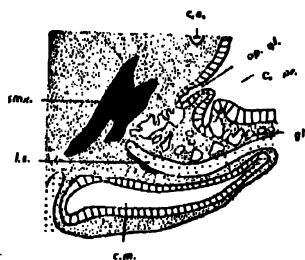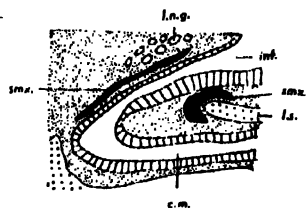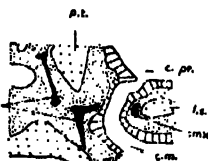
FIG. 2A.   FIG. 2B.   FIG. 2C.

Trans-sections in the region of the septomaxillary bone of *R. cyanophlyctis*.

(Abbreviations as under Fig. 1.)

a few sections on the lateral aspect of the planum terminale (Fig. 2C, *p.t.*). In *R. curtipes* the bone is feebly developed and is disposed on the laminal cartilages as in *R. hexadactyla*.

In the anterior region of *R. grayi*, du Toit (1933) describes the occurrence of a small prechoanal sac in the roof of the mouth. The sac is, however, absent from *R. hexadactyla*, *R. cyanophlyctis* and *R. curtipes*.

The next structure to engage our attention is the eminentia. The eminentia olfactoria in the Ranid forms is usually flat. This feature is noticed in the European form (Gaupp, 1904), in *R. grayi* (du Toit, 1934) and in some South Indian Ranid genera (author, 1934). In *R. hexadactyla* and *R. cyano-phlyctis* the eminentia is elevated on account of the fact that the solum projects into the eminentia in the form of a large supporting cartilage (Figs. 3A
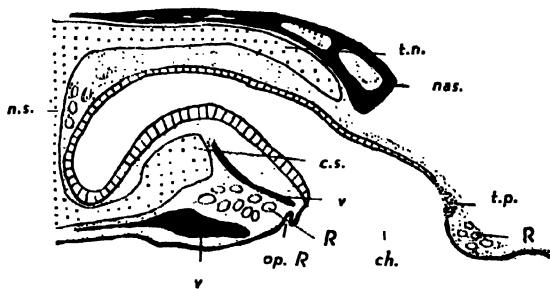
FIG. 3A.

and B, *c.s.*). In *R. curtipes*, on the other hand, there is no supporting cartilage and therefore, the eminentia is flat (Fig. 3C. *c.s.*). It is usually surmised that in Anuran forms which have adapted themselves largely to a terrestrial life, the eminentia is high or elevated. This view is borne out by examples like *Kaloula*, *Microhyla*, *Cacopus* (Euperodon) (author, 1932), *Glyphoglossus* (author, 1932 *a*), *Phrynomerus* (de Villiers, 1930), *Cacosternum* (de Villiers,
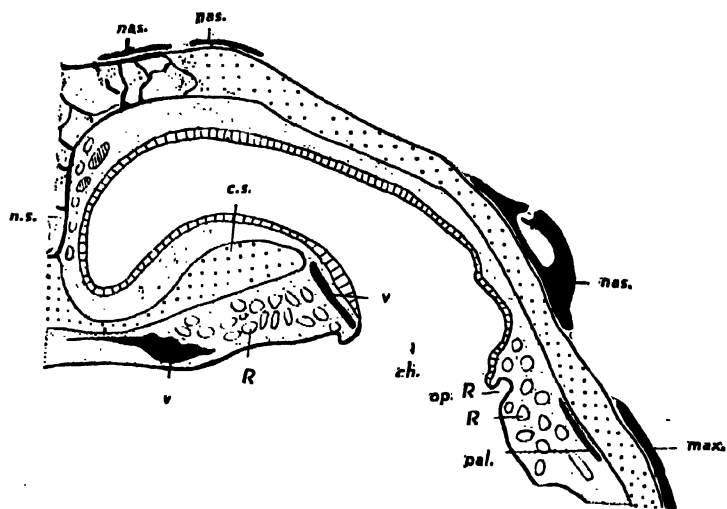
FIG. 3B.
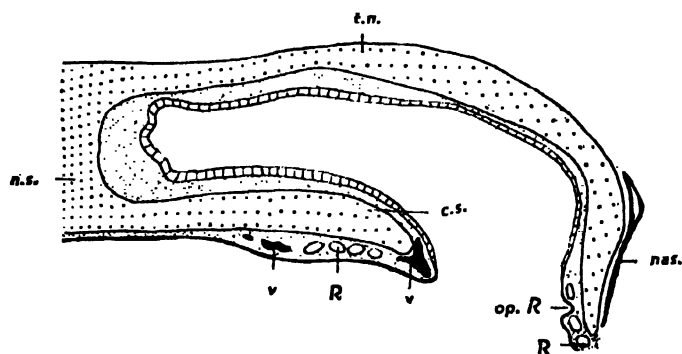
FIG. 3C.

Figs. 3 A, B and C.--Trans-sections in the region of choana of *R. hexadactyla*, *R. cyanophlyctis* and *R. curipes* respectively.
(Abbreviations as under Fig. 1.)

1931), *Breviceps* (de Villiers, 1931 *b*) and *Probreviceps* (de Villiers, 1933). Now, the explanation advanced for the development of an elevated eminentia becomes untenable, since purely aquatic forms like *R. hexadactyla* and *R. cyanophlyctis* have developed it, while it is absent from the other aquatic species, *R. curipes*. Therefore, the elevation of the eminentia has probably nothing to do with terrestrial adaptations of the Anura. It may, however, be said that the structure increases in area purely in response to the sensory requirements of the individual.

A brief reference may be made to the glands occurring in the narial region of the frogs examined by me. Gaupp (1904) describes the disposition

of the glands in the European species of *Rana*. The latest account of the glands in the anuran head is by Müller (1932) who divides the glands under the following three heads :

    (*a*)   the intermaxillary glands,

    (*b*)   the palatal glands (Rachendrüse)

and (*c*)   the tongue glands.

In the South Indian forms the disposition of the glands is almost identical with the description given by Müller. In *R. hexadactyla, R. cyanophlyctis* and *R. curtipes* the intermaxillary glands appear as tripartite structures,— a median and two lateral groups. The lateral ones are situated externally to the premaxilla. Posteriorly, the intermaxillary glands also appear between the superior and inferior cartilages of the premaxilla and therefore, the glandular areas are five in number in this region. The areas again assume a tripartite appearance and finally, the bony separations disappear and the single large intermaxillary gland is seen. The openings of these glands are situated posteriorly and they open into the buccal cavity.

There is a set of glands surrounding the cavum principale (Fig. 2A, *gl.*), being disposed on the dorsal aspect of the lamina superior and they open by several long ducts into the cavum principale (Fig. 1A, *op.gl.*). They are very prominent in *R. hexadactyla* and *R. cyanophlyctis* and are not at all developed in *R. curtipes*. These glands of the cavum principale disappear at the region where the cavum medium gives off the ductus nasolacrimalis. The glandulæ nasalis medialis make their appearance in the anterior region where the fenestra nasobasalis appears in sections. The intermaxillary glands situated ventrally to the fene tra, sometimes extend into the fenestra and surround the recessus medialis of the cavum inferius as in *Acris, Rhacophorus* and other forms (Müller, 1932). In *R. curtipes*, the intermaxillary glands penetrate through the fenestra into the recessus medialis region of the cavum inferius. In *R. hexadactyla* and *R. cyanophlyctis* the penetration of the gland through the fenestra is not noticed. The glandulæ nasalis lateralis (Figs. 1 and 2, *l.n.gl.*) and the Rachendrüse (see Figs. 3A, B and C, *R.*) are disposed as in the European form. Like the other glandular areas, the Rachendrüse are very poorly developed in *R. curtipes*.

The mundwinkeldrüse was for the first time described by de Villiers in *Anhydrophryne* (1931 *c*). Fuchs (1931) described the same gland in some amniotes (*Podocnema expansa*) and some amphibian examples (Bombinator, Dondrobates) under the name of "bursa oris angularis". In *Rana grayi* the occurrence of the gland has been noticed by du Toit (1933). Müller (1932) also refers to this gland in his paper and remarks that it is not a typical gland, but only an accumulation of nuclei. In *R. hexadactyla* alone the

gland is very well developed. The gland, as in the other forms of Anura examined, makes its appearance between the maxilla (Fig. 4A, *max.*) and
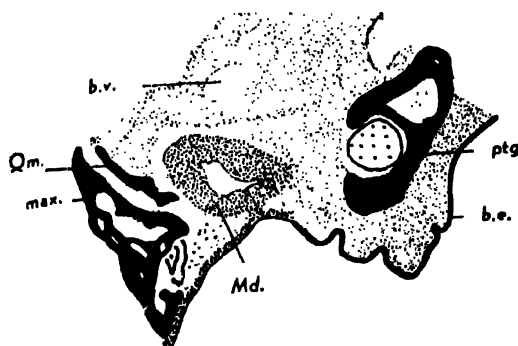
FIG. 4A.

Trans-section showing the mundwinkeldrüse of *R. hexadactyla*.
(Abbreviations as under Fig. 1.)

the pterygoid (*ptg.*) and has a very small lumen; in close association with this, blood and lymph vessels are noticed. Posteriorly, it opens into the buccal cavity. In the region where the mundwinkeldrüse leads into the buccal cavity, the quadratomaxillary (*Qm.*) is also seen. In *R. cyano-phlyctis*, on the other hand, the gland makes its appearance as two or three irregularly scattered areas of lymphocytic aggregations dorsally to the maxilla and pterygoid (Figs. 4B and C, *Md.*); in association with these lymphocytic aggregations, blood and lymph vessels and nerves are seen. It is extremely difficult to identify this structure as a "mundwinkeldrüse", but for the relative positions occupied by the lymphocytes, blood vessels and lymph channels (see Figs. 4B and C). Moreover, there is no opening of these lymphocytic areas into the buccal cavity. Perhaps, Müller's view of the non-glandular nature of the mundwinkeldrüse is largely borne out by *R. cyanophlyctis*. In *R. curtipes* a different state of affairs is met with. A transversely elongated aggregation of lymphocytes, makes its appearance between the maxilla (Fig. 4D, *max.*) and the pterygoid (*ptg.*). This trans-verse elongation is largely due to the presence of a worm parasite (*w.p.*) occupying the space between the lower portion of the eye and the maxilla. The parasite is found on either side in identically the same place and is surrounded by a large number of chitinous cysts. The gland, however, opens into the buccal cavity by a small duct. I am unable to say at present what exactly the function of the gland is like.

I shall make here a brief reference to the other lymphocytic areas (tonsillar patches) found in the anterior region of the cranium. The subject of Batrachian tonsils has been studied by a large number of workers like
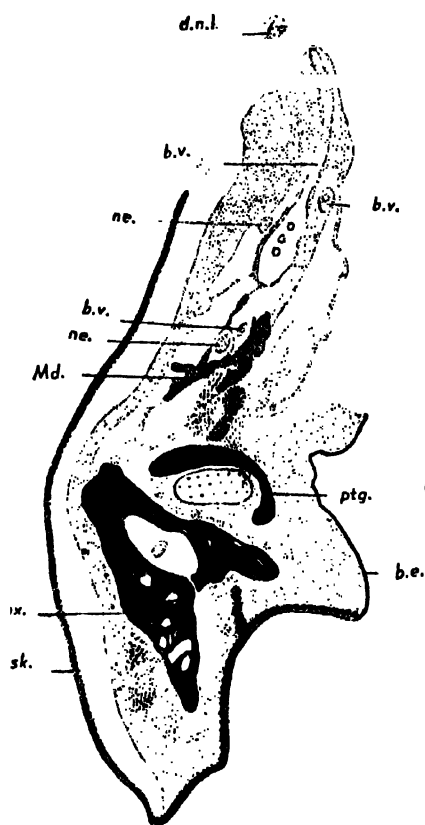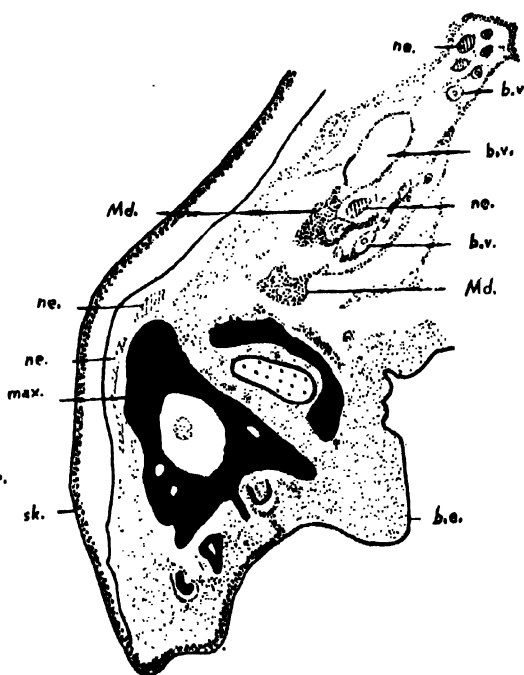
FIG. 4B.   FIG. 4C.

Trans-sections in successive regions of the mundwinkeldrüse of *R. cyanophlyctis.*
(Abbreviations as under Fig. 1.)

Kingsbury (1912), Jolly (1919)* and Myers (1928).   Discussing the structure of the Anuran tonsils, Kingsbury (1912) notes that in essentials the structural features of the Anuran tonsils resemble those of mammals, though at the end he cautions by saying that the "homologization of the amphibian tonsils with those of other groups is regarded as unsafe."   He describes in detail the structure of the proglottidean, lateral and sub-lingual adenoid patches. Myers (1928) referring to Jolly's work (1919) mentions that in the examples investigated by the latter author, "lymphoepithelial" masses are found only in *R. temporaria* and palatine tonsils in both *R. esculenta* and *R. temporaria.*

In *R. hexadactyla* and *R. cyanophlyctis* a large number of lymphocytic aggregations are noticed and *R. curtipes* is peculiarly free from these adenoid
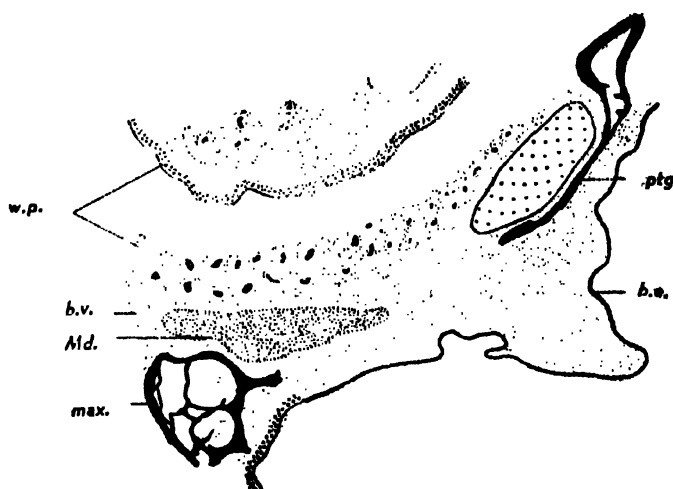
FIG. 4D.

Trans-section of the mundwinkeldrüse of *R. curtipes*.

(Abbreviations as under Fig. 1.)

patches. In *R. hexadactyla* and *R. cyanophlyctis* the numerous adenoid patches are disposed in the roof of the mouth, on the tongue and below it. The areas that are found just below the tongue and also far away from it (*i.e.*, those that are situated above the mandible) are all labelled by Myers as sublingual tonsils. More correctly, the ones situated just below the tongue should be called sublingual and the others nearer to the mandible must be labelled mandibular tonsils. Besides the areas described above where these tonsillar patches occur, lymphocytic aggregations also occur in the region of the cavum inferius. One pair of these tonsils opens into the cavum inferius and the other only surrounds the maxillary end of the cavum. In the two examples referred to above there is also a large adenoid area just below each eye. These may be designated the subocular tonsils.

The Membrane Bones of the Anterior Region.

Included under the membrane bones of the olfactory region are the premaxillæ, maxillæ, vomer (prevomer), palatine and septomaxillary. A reference has already been made to the septomaxillary bone. The premaxillæ, maxillæ, vomer (prevomer) and palatine are represented as in the European form *Rana fusca* (Gaupp, 1904). In all the species of *Rana* examined by me, the lateral squame of the premaxilla is longer than the median one. The premaxilla, maxilla and vomer (prevomer) are dentigerous. The nasal bone is disposed uniformly in all the species of *Rana* studied. Anterior to the planum terminale the nasal bones invest the tectum and

posterior to the planum they protect the cavum principale. In the antorbital region, investing the dorsal aspect of the antorbital cartilage is noticed the posterior portion of the nasal (Figs. 3A, B and C, *nas.*) ; the bone is thick and muscles are inserted into it.

The Ethmoidal Region.

The sphenethmoid (os en ceinture, Cuvier) is a girdle-shaped bone which may or may not be divided into a right and left half by means of a median
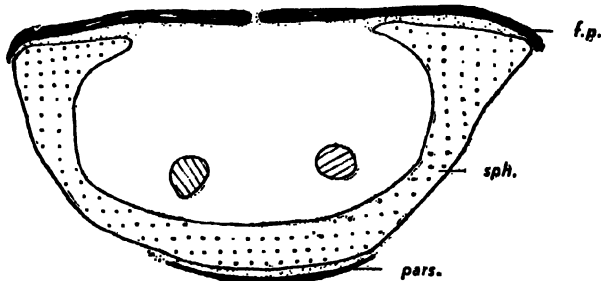
FIG. 5A.
The sphenethmoid of *R. curtipes*.
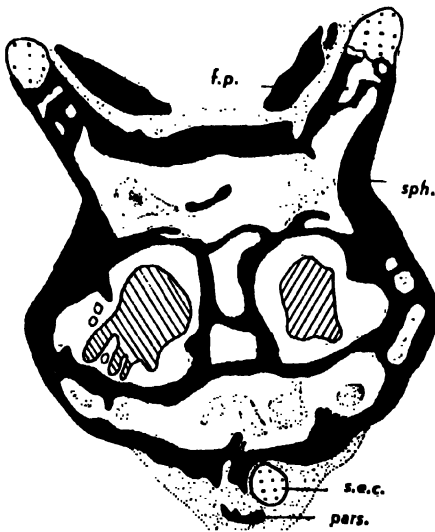
FIG. 5B.

FIG. 5C.

Anterior and posterior sphenethmoidal regions of *R. cyanophlyctis*.
(Abbreviations as under Fig. 1.)
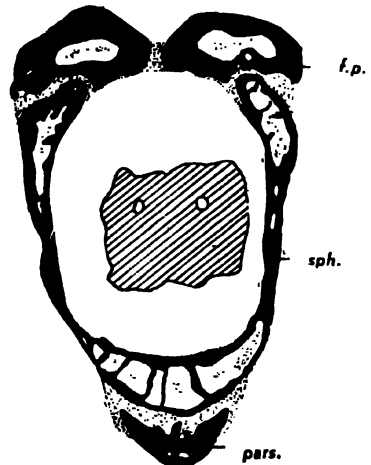
cartilage. In some examples, however, the ethmoidal region is not at all ossified. To this latter category belongs *R. curtipes* (Fig. 5A). In *R. cyanophlyctis*, on the other hand, the sphenethmoid is very well developed

and is not separated ventrally into lateral halves by a median cartilage. In sections in the anterior ethmoidal region, the occurrence of a small piece of cartilage (Figs. 5B and C, *s.e.c.*) between the sphenethmoid (*sph.*) and parasphenoid (*pars.*) is noticed, whose exact significance, it is not possible to say. This subethmoidal cartilage (*s.e.c.*) is also noticed in *Rana grayi* (du Toit, 1933). In *R. hexadactyla* the sphenethmoid is well ossified as in
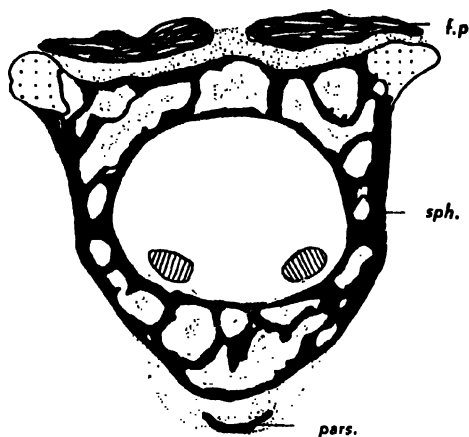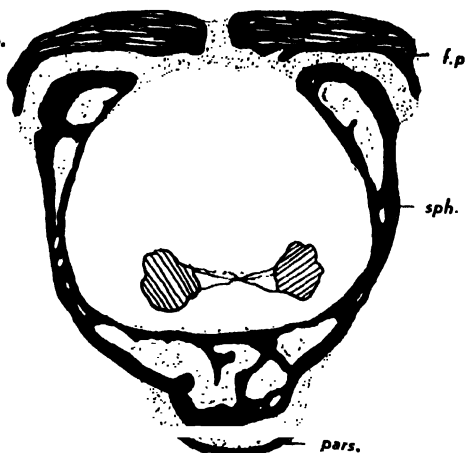
FIG. 5D.   FIG. 5E.

Anterior and posterior sphenethmoidal regions of *R. hexadactyla*.
(Abbreviations as under Fig. 1.)

R. cyanophlyctis. The frontoparietals in this region are thick and massive as in *R. hexadactyla* and *R. cyanophlyctis*; in *R. curtipes* the bones are thin.

The Otic Region.

The otic region in all the three species of *Rana* that I have examined follows a common plan. The paraquadrate (squamosal) bone is the first to make its appearance in sections and posteriorly to it the middle ear and annulus tympanicus are seen. In the region where the middle ear makes its appearance in *R. hexadactyla*, the annulus tympanicus with the ventrally situated maxilla and quadrato-maxillary are also seen. This extension of the maxilla posteriorly seems to be a common condition among the Ranids and it is also noticed in *Rana grayi* (du Toit, 1933). The membrane bones and the associated cartilages in the middle ear region are drawn in the series of Figs. 6A, B and C. It will be noticed that the arrangement of bones and cartilages are not essentially the same as the descriptions given either for European species of *Rana* or the South African form, *Rana grayi* (du Toit, 1933). In *R. hexadactyla*, in the region where the prootic bone has not yet

made its appearance the middle ear, annulus tympanicus and the paraquad-
rate bone are all noticed.   In this region the circular pars externa plectri
(the extrastapedial process) is also present, and therefore, it appears more
anteriorly than it is noticed in the other Ranids.   In posterior sections,
the transitional cartilage (Fig. 6A, *tr.c.*) which appears internally to the
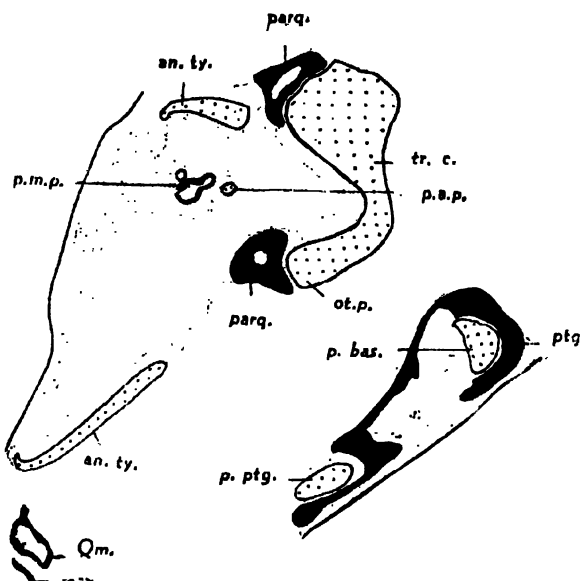
FIG. 6A.
(Abbreviations as under Fig. 1.)

paraquadrate (*parq.*) unites with the otic process (*ot.p.*) and the cristal end
(Fig. 6B, *c.tr.c.*) of this combined cartilage unites with the ossified otic capsule
posterior to figure 6B.   Moreover in Fig. 6A, it is noticed that a small an-
terior portion of the processus basalis (*p.bas.*) also appears being invested
by the pterygoid (*ptg.*).   Peculiarly, however, in posterior sections the
processus basalis (Fig. 6B, *p.bas.*) which arises from the processus oticus
(*ot.p.*) is partially invaded by the pterygoid (*ptg.*).   Both in 6A and B,
the pars media is seen in close association with a cartilage which is the pars
ascendens plectri.   The pars ascendens plectri establishes a connection
between the circular pars externa plectri on the one hand, and the
cartilaginous ventromedial portion of the crista on the other. The occurrence
of the maxilla and the quadratomaxillary in close association with the
ventral portion of the annulus tympanicus are also shown in Figs. 6A and B.
In Fig. 6C, where the eustachian tube opens into the buccal cavity, the
ossified otic capsule (*o.c.*) is noticed and this gives rise ventrally to a large
cartilaginous projection (*c.p.ot.*), which gives articulation to the posterior
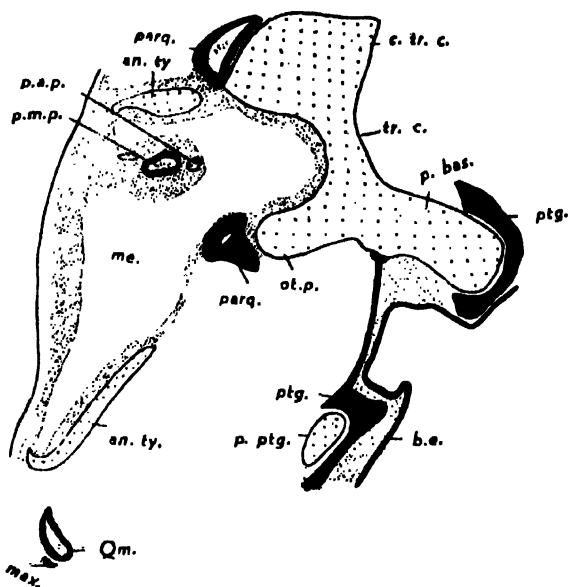
FIG. 6B.
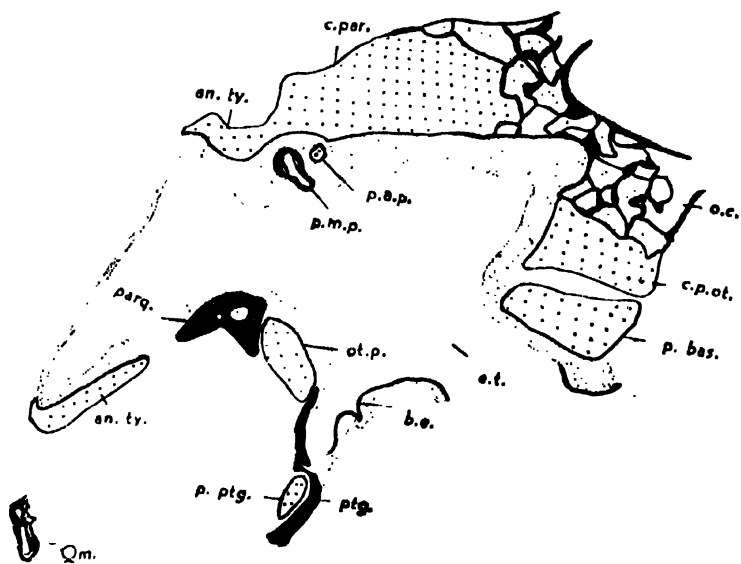
FIG. 6C.

Consecutive trans-sections in the middle ear region of *R. hexadactyla*.

(Abbreviations as under Fig. 1.)

portion of the processus basalis (*p.bas.*). The pars ascendens plectri (*p.a.p.*) is seen very close to its place of attachment with the crista. The dorsal

portion of the annulus tympanicus is also seen in the same figure as a projection from the crista (*c.par*).   The quadratomaxillary is noticed ventrally to the ventral portion of the annulus tympanicus.

In the opercular region there is a small pars interna plectri and the operculum (stapes) is saucer-shaped.   It depends from the upper part of the cartilaginous otic capsule.   Posterolaterally, it has a knob for the attachment of a muscle.

In the suspensorial region the quadratomaxillary invades the quadrate cartilage and the paraquadrate extends laterally over the quadratomaxillary. No fusion between the paraquadrate and the quadratomaxillary is noticed as in one of the specimens of *Rana grayi* studied by du Toit (1933).

In *R. cyanophlyctis*, as in *R. hexadactyla*, even before the appearance of the prootic bone, the pars externa plectri which is a rounded cartilage makes its appearance.   The processus basalis is not met with in this region.
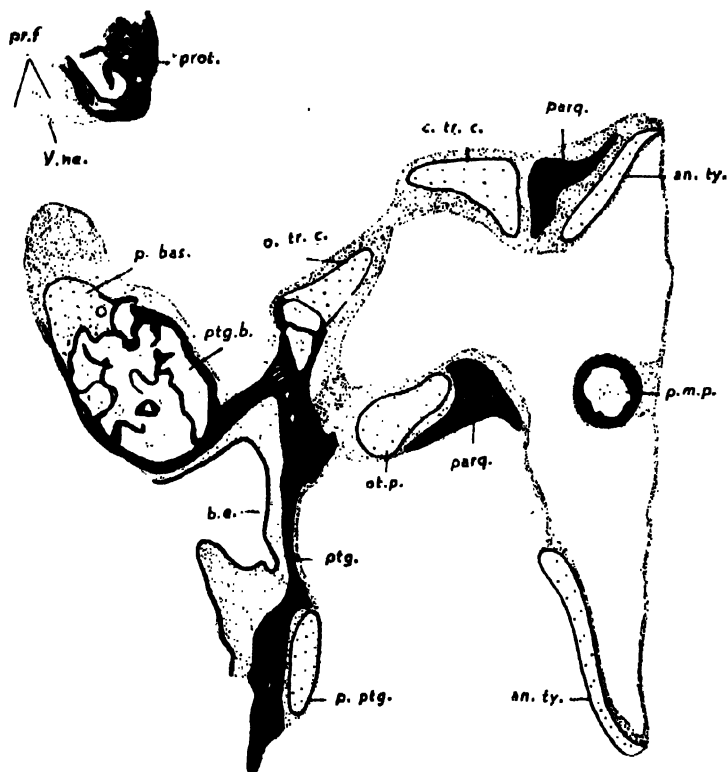
FIG. 7.

Trans-section in the processus basalis region of *R. cyanophlyctis*.
(Abbreviations as under Fig. 1.)

In a slightly posterior region, the transitional cartilage unites with the processus oticus as in *R. hexadactyla* and the cristal part (Fig. 7, *c.tr.c.*) of this united cartilage fuses with the ossified extension of the otic capsule posterior to Fig. 7. The processus basalis (*p.bas.*) makes its appearance anterior to the opening of the eustachian passage, being invaded by the pterygoid (*ptg.*) and it is noticed that the cartilaginous portion of the processus basalis is considerably minimised. Moreover the pterygoid also invades the extension (*o.tr.c.*) of the oticus cartilage (*ot.p.*). At the region where the eustachian passage opens into the buccal cavity, the processus basalis invaded by the pterygoid bone, is noticed to articulate with a projection from the bony otic capsule. This condition of the articulation of the basal process is also met with in *R. hexadactyla*. In the same region on the left side of the animal, the bony pars media plectri gives rise to a small cartilaginous portion towards the paraquadrate, which in posterior sections disengages itself from the columella and disappears from the sections. This cartilaginous piece, perhaps, represents the pars ascendens plectri which is so prominently noticed in *R. hexadactyla*. The pars interna becomes manifest in posterior sections only.

The operculum is saucer-shaped and depends from the bony otic capsule. Posterolaterally there is a small knob for the insertion of a muscle.

The suspensorial region is as in *R. hexadactyla*. In *Rana cyanophlyctis* the quadratomaxillary invades the quadrate cartilage almost completely.
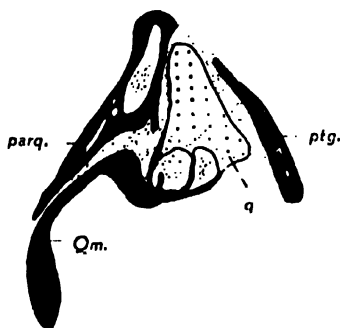
Fig. 8.

The suspensorial region of *R. cyanophlyctis.*

(Abbreviations as under Fig. 1.)

Again, an extension of the paraquadrate over the quadratomaxillary is met with, as in *R. hexadactyla*.

In *R. curtipes*, where the paraquadrate and the middle ear appear in sections, the quadratomaxillary is not visible. The crista (Fig. 9A, *c.par.*)

is very prominent and is cartilaginous. The processus basalis, however, has not yet made its appearance. Long before the appearance of the basal process, the united transitional (*tr.c.*) and processus oticus (*ot.p.*) cartilage establishes a connection with the crista (*c.par.*). Thus in this case the oticus connection of the palatoquadrate with the cranium is very short; in *R. hexadactyla* and *R. cyanophlyctis*, on the other hand, the connection is lengthened. This is on account of the fact that in the latter two examples the crista is an extremely long cartilaginous piece while in *R. curtipes*, it is highly abbreviated in length. Posteriorly, the processus pterygoideus (Fig. 9A, *p.ptg.*) cartilage fuses with the oticus cartilage (*ot.p.*) and the large
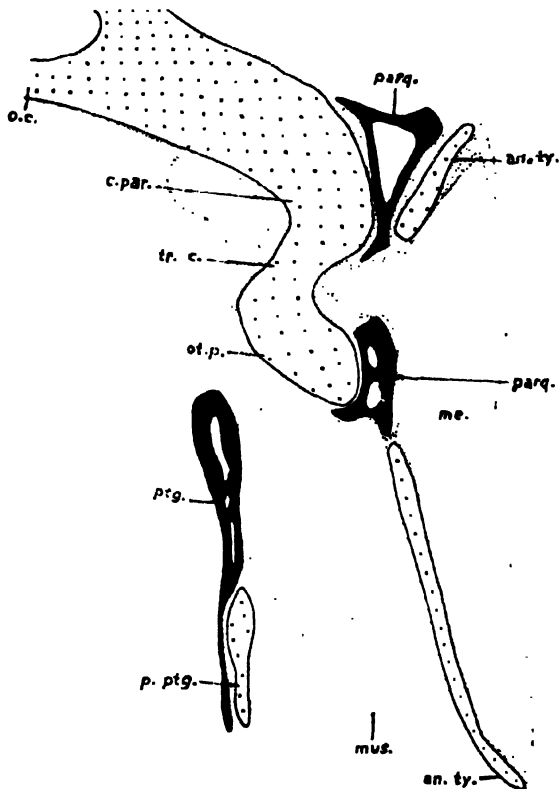
FIG. 9A.

processus basalis (Fig. 9B, *p.bas.*) is given off from this. It is noticed th a a part of the processus basalis cartilage is invaded by the pterygoid bone as in *R. hexadactyla* and *R. cyanophlyctis*. In Fig. 9C, the pars externa plectri is drawn and this is the first to make its appearance of the plectral

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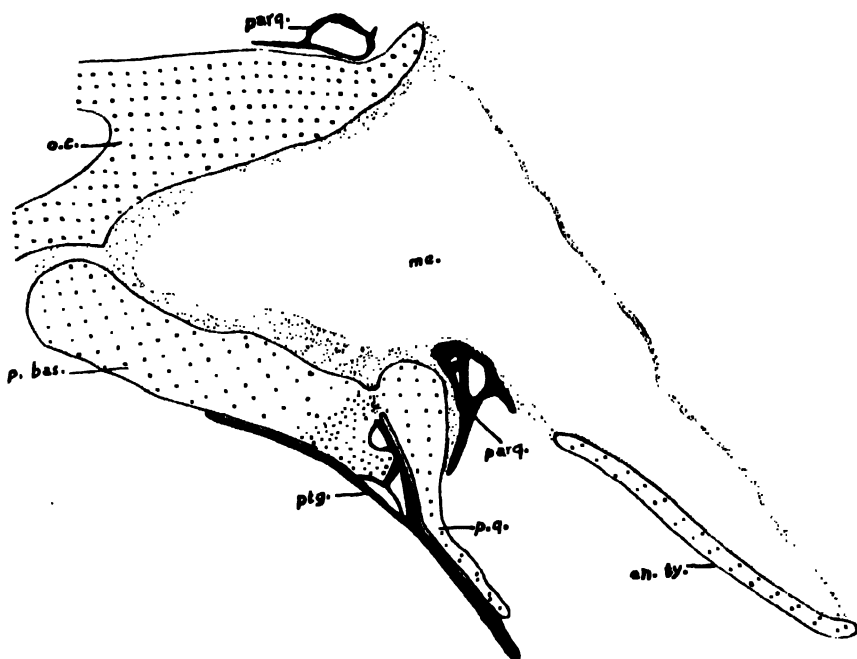
FIG. 9B.
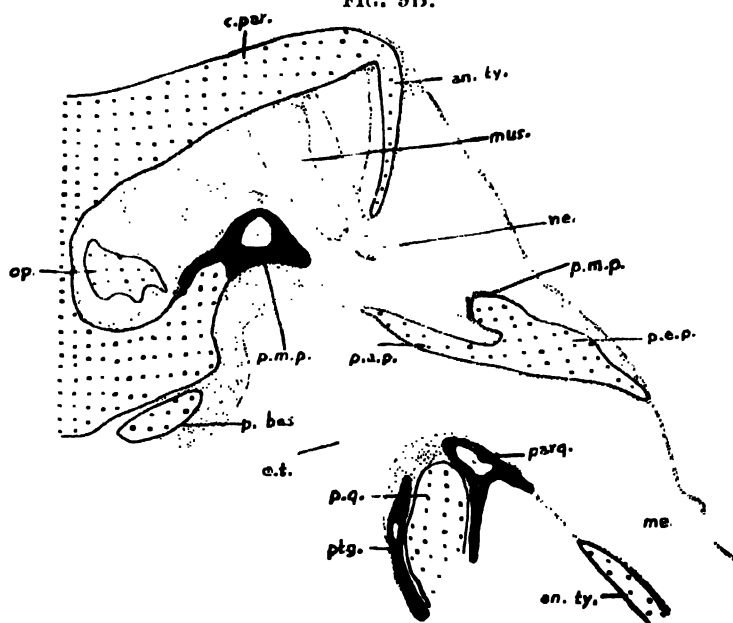
FIG. 9C.
Consecutive trans-sections of the middle ear region in *R. curtipes*.
(Abbreviations as under Fig. 1.)

apparatus. The large pars externa (*p.e.p.*) gives rise internally to a pars ascendens plectri; this ascendens cartilage fuses with the crista, as in *R. hexadactyla*. The pars media is only superficially ossified with a feeble core of cartilage, thereby exhibiting the cartilaginous origin of the columella. The dorsal portion of the annulus tympanicus extends from the crista (*c.par.*) as in *R. cyanophlyctis*. It is obvious from the figure that there is no projection from the ventral portion of the cartilaginous otic capsule for the articulation of the posterior part of the processus basalis (*p.bas.*) as in *R. hexadactyla* and *R. cyanophlyctis*.

The small operculum which is crescentic in appearance depends from the upper wall of the otic capsule, and posterolaterally possesses a knob for the insertion of a muscle. The fenestral opening is small.

The suspensorial region is as previously described for *R. hexadactyla*. The invasion of the quadrate cartilage by the quadratomaxillary is very feeble in *R. curtipes*.

Summary.

1.   The plica obliqua depends from the cartilago obliqua and not from the tectum nasi.

2.   A recessus sacciformis is not present in the forms described.

3.   A prechoanal sac is absent.

4.   The shape of the septomaxillary in *R. hexadactyla* and *R. curtipes* differs from the same in *R. cyanophlyctis*.

5.   The eminentia olfactoria is elevated in *R. hexadactyla* and *R. cyanophlyctis* and not in *R. curtipes*.

6.   An unconnected piece of cartilage occurs in *R. cyanophlyctis* below the sphenethmoid.

7.   The sphenethmoid is bony in *R. cyanophlyctis* and *R. hexadactyla*, whereas in *R. curtipes*, it is cartilaginous.

8.   The "mundwinkeldrüse" in *R. hexadactyla* and *R. curtipes* are similar in structure; in *R. cyanophlyctis* the gland differs in shape and does not possess a lumen. It also does not open into the buccal cavity.

9.   In *R. hexadactyla* and *R. cyanophlyctis* there is a large cartilaginous projection from the ventral portion of the otic capsule with which the posterior portion of the processus basalis articulates.

10.   The crista parotica is very short in *R. curtipes*, while in *R. hexadactyla* and *R. cyanophlyctis*, it is comparatively long.

11.   The pterygoid bone, in all the three species of *Rana* examined, invades the processus basalis partially.

12. A pars ascendens plectri which is a commissural cartilage between the crista and the pars externa plectri, is observed only in *R. hexadactyla* and *R. curtipes*.

13. The quadrate cartilage is feebly invaded by the quadratomaxillary bone in *R. curtipes*.

14. Since the operculum depends from the upper wall of the otic capsule, its movement must be considerably diminished in all the three species of *Rana* examined.

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THREE NEW SPECIES OF TREMATODES FROM BIRDS.

By S. S. Patwardhan, M.Sc.,

Department of Zoology, College of Science, Nagpur.

THE three new species of Trematodes described in this paper were all obtained from birds dissected by me in the Zoology Laboratory from time to time. Numerous specimens of *Lyperosomum* Looss, 1899, were found in the gall bladder of a Black-headed Maina *Temenuchus pagodarum* (Gm.). Similarly several specimens of *Proalaria* La Rue, 1926, were obtained from the intestine of a King Fisher, *Alcedo atthis* Linn. Only three specimens, however, of *Neodiplostomum* La Rue, 1926, were collected from the intestine of a Barn Owl, *Tyto alba stertens* Hartest.

1. *Lyperosomum colorosum* n. sp.

Anatomy :—A large number of specimens were found in the gall bladder of a Black-headed Maina *Temenuchus pagodarum* (Gm.) at Nagpur, C. P., India. The worms are elongated, cylindrical, tapering at both ends and measure 1·7 to 3·0 mm. in length and 0·192 to 0·34 mm. in maximum width which is found at the level of acetabulum. Examination of the living worms under a microscope showed that the forebody is flexed dorsally and this makes the acetabulum very prominent. The integument is smooth and a large number of pigmented particles lie scattered in the parenchyma throughout the body. The oral sucker (Fig. 1. *o.s.*) is subterminal, elliptical and measures 0·076 to 0·134 mm. ×0·092 to 0·16 mm. The muscular pharynx is spherical and measures 0·043 to 0·075 mm. in diameter. The œsophagus is short and measures 0·06 to 0·1 mm. in length. The intestinal cæca extend backwards as far as the four-fifth of the entire length of the worm. The acetabulum is rounded and is 0·138 to 0·24 mm. in diameter. Its anterior border is 0·356 mm. to 0·63 mm. behind the anterior end of the body. The ratio of the diameters of the oral sucker to the acetabulum is about 1 : 2. The excretory system is typical of the genus.

Both the testes are posterior to acetabulum, situated one behind the other along the median line of the body. The anterior testis is spherical, 0·127 to 0·22 mm. in diameter and 0·081 mm. behind the acetabulum. The

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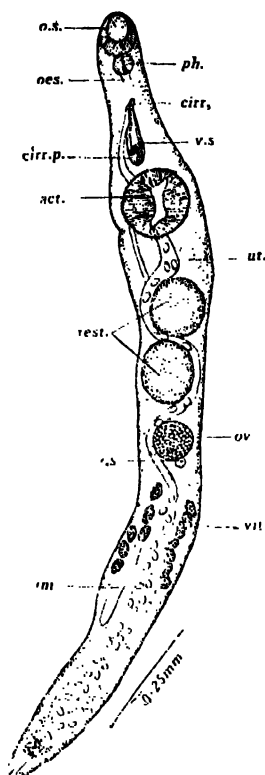
Fig. 1.

posterior testis is slightly oval and is separated from the anterior one by a loop of the uterus which passes between the two testes. The posterior testis measures $0 \cdot 108$ to $0 \cdot 208$ mm. $\times 0 \cdot 123$ to $0 \cdot 217$ mm. The genital pore lies midway between the rim of the acetabulum and the anterior end of the body. The cirrus pouch is elongated, pyriform and measures $0 \cdot 12$ to $0 \cdot 21 \times 0 \cdot 04$ to $0 \cdot 07$ mm. It contains a large vesicula seminalis. The cirrus in some specimens was protruding and is surrounded by a few prostatic cells.

The spherical ovary is smaller than both the testes and is situated $0 \cdot 04$ mm. behind the posterior testis along the median line of the body. It measures $0 \cdot 084$ to $0 \cdot 148$ mm. in diameter. The receptaculum seminis is dorsal and lateral to the ovary and measures $0 \cdot 023$ to $0 \cdot 04$ mm. in diameter. The shell gland is similar in position but slightly ventral. Vitellaria are situated posterior to ovary and are in two lateral rows of six to seven follicles on each side. They commence $1 \cdot 3$ to $2 \cdot 0$ mm. behind the anterior end of the body and extend for about $0 \cdot 25$ mm. along the length of the body. The

uterus extends backwards as far as the posterior end of the body and its coils separate the two testes from one another. Eggs are large, elliptical, thick-shelled and measure in balsam 0·0125 to 0·022 × 0·025 to 0·04 mm.

Discussion.—Paired symmetrical vitellaria, much elongated body and the position of the testes one behind the other place this worm in the genus *Lyperosomum* Looss, 1899, sub-family, *Dicrocœlinœ* Looss, 1899. Following the key given by Skrjabin and Udinzew, 1930, the species described in this paper resembles *L. sinuosum* Travassos, 1917. It however differs from the latter in possessing a considerably smaller body, an elliptical oral sucker, oval posterior testis, vitellaria in two lateral rows of six to seven distinct compact masses and in having in its parenchyma a large number of pigmented particles scattered throughout the body.

Since the publication of the key by Skrjabin and Udinzew, 1930, only one species, *viz.*, *L. microrosalis* Yamaguti, 1933, has been described from *Milvus lineatus lineatus* Grey, from Japan. The species described in this paper differs clearly from *L. micrirosalis* in having smaller suckers and a round ovary, in the ratio of the oral and ventral suckers and other measurements. I, therefore, regard this species as new and propose for it the name *Lyperosomum colorosum*, with the following specific diagnosis :—

Lyperosomum Looss, 1899 : Length 1·7 to 3·0 mm. ; maximum width 0·192 to 0·34 mm. ; forebody shorter than hindbody ; cuticle smooth ; parenchyma with pigmented particles scattered in it ; oral sucker subterminal, elliptical 0·076 to 0·134 mm. × 0·092 to 0·16 mm. ; muscular pharynx 0·043 to 0·07 mm. in diameter ; acetabulum 0·138 to 0·24 mm. in diameter ; anterior testis 0·127 to 0·22 mm. in diameter ; posterior testis sub-spherical, 0·108 to 0·208 mm. × 0·123 to 0·217 mm. ; ovary 0·084 to 0·148 mm. in diameter ; vitellaria in two lateral rows of six to seven distinct follicles ; eggs large, few thick-shelled 0·0125 to 0·22 × 0·025 to 0·04 mm.

Host :—*Temenuchus pagodarum* (Gm.).

Habitat :—Gall bladder.

Locality :—Nagpur, C.P., India.

2. *Proalaria alcedensis* n. sp.

Anatomy.—The body measures 2·24 mm. in length and is divided into two parts. The forebody (Fig. 2) is flattened and roughly four-sided. It measures 0·72 × 0·66 mm. The hindbody is cylindrical, rounded off posteriorly and measures 1·52 × 0·5 mm. The ratio of the lengths of the fore and hind body is about 1 : 2. The oral sucker is round, terminal and measures 0·035 mm. in diameter. On its either side is situated a sucker-like prominence. There is no prepharynx. The pharynx is spherical, muscular
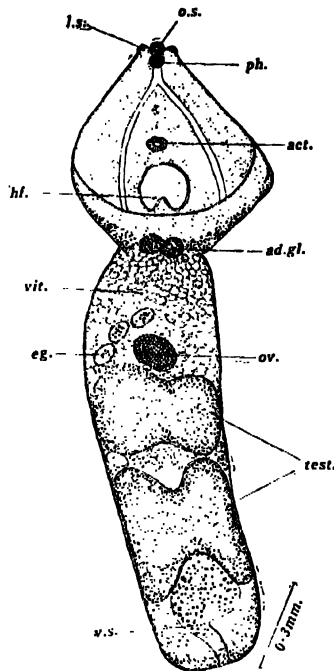
FIG. 2.

and 0·037 mm. in diameter. It is followed by a short œsophagus which forks into two intestinal cæca in front of the acetabulum. The latter is transversely ovate, measures 0·056 × 0·048 mm. and is situated 0·4 mm. behind the anterior end of the body. The holdfast organ is slightly oval and invaginated posteriorly. It measures 0·175 × 0·15 mm. A pair of adhesive glands are situated behind the holdfast organ.

The ovary is transversely oval and is situated considerably behind the junction of the fore and hind body. It measures 0·125 × 0·1 mm. The vitellaria consist of closely packed follicles which are confined to the anterior half of the hindbody. The uterus extends for a short distance in front of the ovary and contains large but few eggs measuring 0·075 × 0·092 mm. The genital pore is situated at the posterior end of the body.

The testes occupy the middle third of the hindbody and fill up the entire width. They are roughly rectangular and have a deep depression on both the anterior and posterior face. The anterior testis measures 0·5 × 0·325 mm. and the posterior testis 0·4 × 0·41 mm. The vesicula seminalis is a voluminous sac-like structure situated behind the posterior testis. The details of the cirrus, vesicula seminalis and pons prostatica could not be made out.

Discussion.—In possessing a sub-circular holdfast organ, lateral suckerlike appendages near the oral sucker and a uterus confined to hindbody, the species described in this paper belongs to the genus *Proalaria* La Rue, 1926. So far there are thirteen species included in this genus. The species described here differs from the following species, *Alaria gavium* Guberlet, 1922, *Hemistomum excavata* Rud. Dies., *H. confusum* Krause, 1925, *H. spathacæum* Rud. Dies., *H. trilobum* Rud. Dies., *P. huronensis* La Rue, 1927, *P. variabilis* Chandler, 1932, *P. butasturia* Tubangui, 1932, *P. mergi* Yamaguti, 1933, in the ratio of the fore and hind body and other important characters. It differs from *H. intermedium* Johnston, 1904, and *H. triangulare* Johnston, 1904, in the shape, size and disposition of the gonads and other measurements. It also differs from *A. indistincta* Guberlet, 1922 in the absence of the receptaculum seminis. Description of *P. clavata* Ciurea, 1928, was not available to me in India. I conclude, therefore, that it is a new species which I name *Proalaria alcedensis* with the following specific characterisation :

Proalaria La Rue, 1926 : Total length 2·24 mm. ; forebody 0·72 × 0·66 mm. ; hindbody 1·52 × 0·5 mm. ; oral sucker subterminal 0·035 mm. in diameter ; pharynx 0·037 mm. in diameter ; acetabulum ovate, 0·056 × 0·48 mm.; holdfast organ elliptical invaginated posteriorly, 0·175 mm. × 0·15 mm. ; ovary ovate, behind the junction of fore and hind body, 0·125 × 0·1 mm. ; vitellaria confined to anterior half of the hindbody, anterior and posterior testes rectangular, deeply depressed on the anterior and posterior face ; anterior testis 0·5 mm. × 0·325 mm. ; posterior testis 0·4 mm. × 0·41 mm. ; vesicula seminalis, a voluminous sac behind the posterior testis ; uterus extends slightly in front of the ovary ; eggs, few, large thick-shelled and 0·075 × 0·092 mm.

Host :—King Fisher *Alcedo atthis* Linn.

Habitat :—Intestine.

Locality :—Nagpur, C.P., India.

3. *Neodiplostomum tylense* n. sp.

Anatomy.—The body measures 2·82 mm. in total length and is distinctly divided into two unequal regions. The forebody (Fig. 3) is flattened and measures 1·62 × 1·23 mm. Its lateral margins unite posteriorly to form a spoon-shaped depression. The hindbody is cylindrical and measures 1·2 × 0·63 mm. The cuticle is smooth. The smaller oral sucker is not very prominent and measures 0·056 mm. in diameter. The pharynx is small, globular and 0·062 mm. in diameter. It is followed by a short œsophagus which forks into a pair of intestinal cæca in front of the acetabulum. The acetabulum is 0·087 mm. in diameter and is situated 0·64 mm. behind the
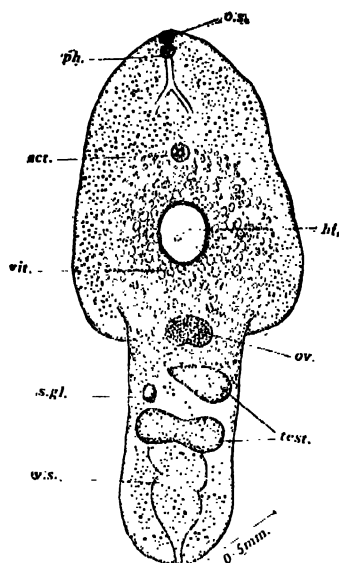
FIG. 3.

anterior end of the body. The holdfast organ is sub-circular, small, 0·294 mm. in diameter and is 0·22 mm. behind the ventral sucker.

The ovary is situated in front of the testes at the junction of the fore and hind body. It is a compact ovate mass measuring 0·25 × 0·19 mm. The uterus and eggs are not developed. The vitellaria consist of a large number of small follicles scattered around the holdfast organ and extend into the anterior third of the hindbody. The shell gland is situated near the left side at a level between the two testes.

The testes are situated one behind the other and occupy the middle third of the hindbody. The anterior testis is pear-shaped, slightly to the left and measures 0·375 × 0·225 mm. The posterior testis is deeply bilobed, large and measures 0·45 × 0·225 mm. Vesicula seminalis is a large sac-like structure situated behind the posterior testis. The genital pore is situated at the posterior end of the body. Other details could not be made out.

Discussion.—The species described in this paper belongs to the genus *Neodiplostomum* La Rue, 1926, because of the presence of a body divided into two parts, lateral margins of the forebody meeting posteriorly, shell gland at a level between the two testes, absence of lateral suckerlike appendages and of suckers from the dorsal surface. There are so far nineteen species included in this genus. The present species differs from the following species, in the ratio of the forebody and hindbody and other characters : *Diplostomum spathula*

Brandes, *D. longum* Brandes, *D. siamense* Poirier, *D. bifurcatum* Wedl Brandes, *Hemistomum cochleare* Krause, 1915, *H. ellipticum* Brandes, *Neodiplostomum poirieri* Dubois, 1932, *N. grande* Dies. Krause, and *N. kashmiranum* Faust, 1927. It differs from *D. spathulaforme* Brandes, *N. gavialis* Dharam Narain, 1930, in the absence of papillæ around the holdfast organ and the disposition of the gonads, from *N. pseudattenuatum* Dubois, 1927 and *H. attenuatum* v. Linst., 1906, in the shape, size and disposition of the gonads, and from *N. lucidum* La Rue, 1927 in the absence of cuticular spines. The descriptions of the five species, *H. auritum* Duj, 1845, *N. perlatum* and *N. cuticola* Ciurea, 1930, *N. marchelloides* and *N. fungiloides* Semenow, 1927, are not available to me in India. I consider the species described in this paper as new and name it *Neodiplostomum tytense* with the following specific diagnosis :

Neodiplostomum La Rue, 1926 : Total length 2·82 mm. ; forebody 1·62 × 1·23 mm. ; hindbody 1·2 × 0·61 mm. ; ratio of the forebody to the hindbody 1·35 : 1 ; oral sucker 0·056 mm. in diameter ; pharynx 0·062 mm. in diameter ; acetabulum 0·087 mm. in diameter and 0·64 mm. behind the anterior end of the body ; holdfast organ 0·294 mm. in diameter and 0·22 mm. behind the ventral sucker ; ovary at the junction of fore and hind body 0·25 × 0·19 mm. ; vitellaria around the holdfast organ and in the anterior third of hindbody ; anterior testis pear-shaped, slightly to the left, 0·375 × 0·225 mm. ; posterior testis deeply bilobed 0·45 × 0·225 mm. ; vesicula seminalis behind the posterior testis ; genital pore at the posterior end of the body.

Host :—Tyto alba sterlens Hartest.

*Habitat :—*Intestine.

*Locality :—*Nagpur, C.P., India.

EXPLANATION OF FIGURES.

Fig. 1.—*Lyperosomum colorosum* n.sp. Ventral view.
Fig. 2. *Prealaria alcedensis* n.sp. Ventral view.
Fig. 3.—*Neodiplostomum tytense* n.sp. Ventral view.

REFERENCE LETTERS.

act.	..	Acetabulum.	*o.s.*	..	Oral sucker.
ad.gl.	..	Adhesive glands.	*ov.o.*	..	Ovary.
cirr.	..	Cirrus.	*ph.*	..	Pharynx.
cirr.p.	..	Cirrus pouch.	*r.s.*	..	Receptaculum seminis.
hf.	..	Holdfast organ.	*s.gl.*	..	Testes.
int.	..	Intestinal caecum.	*test.*	..	Shell gland.
l.s.	..	Lateral suckers.	*vit.*	..	Vitellaria.
oes.	..	Oesophagus.	*v.s.*	..	Vesicula seminalis.

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A SECOND SPECIES OF *PROCAMALLANUS* BAYLIS 1923 FROM INDIA.

BY R. B. KULKARNI, M.Sc.,

Department of Zoology, College of Science, Nagpur.

THE nematodes described in this communication were obtained from the intestine of a Silurid fish, *Clarias batrachus* Bl. The material consisted of six worms of which four were females and two males.

Anatomy.

The male measures $3 \cdot 85$ mm. in length and $0 \cdot 061$ mm. in maximum width; the female measures $5 \cdot 07$ to $7 \cdot 26$ mm. in length and $0 \cdot 079$ mm. in maximum breadth. The anterior end is narrow and rounded. The cuticle is smooth and unstriated. The buccal capsule (Fig. 1, *B.C.*) measures $0 \cdot 082$ mm. in length and $0 \cdot 061$ mm. in diameter. It has a continuous smooth chitinous lining without leaf crowns or ridges. Two knoblike structures (*K.*) are present at the base of the buccal capsule. The œsophagus measures $0 \cdot 41$ mm. in length
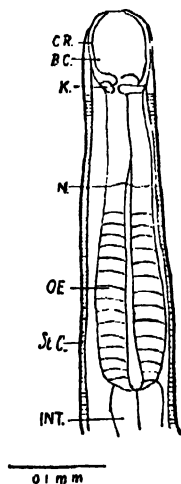
FIG. 1.
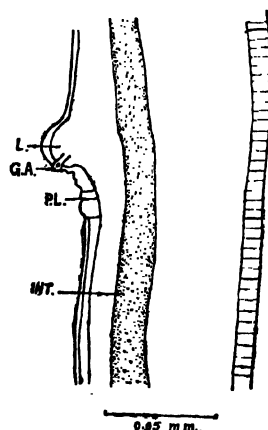
FIG. 2.

from the anterior end. The nerve ring and the excretory pore are situated $0 \cdot 24$ mm. and $0 \cdot 31$ mm. respectively from the anterior end of the body.

29

The caudal end of the female (Fig. 5) is drawn out, bluntly pointed at the apex and measures 0·11 mm. in length. The vulva is situated at about the middle of the body 1·4 to 3·38 mm. in front of the posterior end of the body. A distinct lobelike structure forms the anterior lip of the vulva. The vagina is directed posteriorly.

FIG. 3.

FIG. 5.
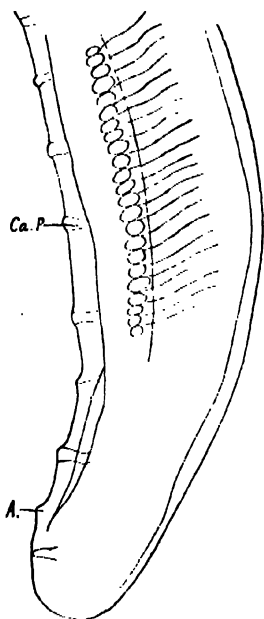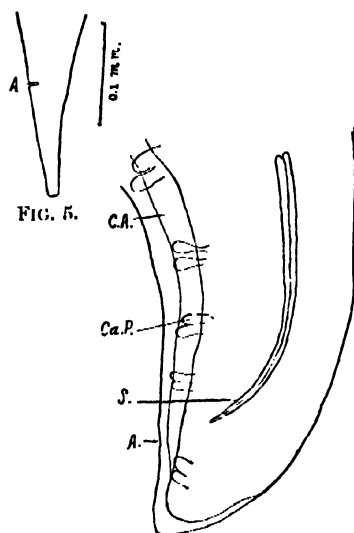
0·05 m.m.

FIG. 4.

The caudal end of the male is slightly curved ventrally and is bluntly rounded at the tip. The caudal alae are well developed. There are seven pairs of pre-anal and one pair of post-anal pedunculated papillae (Fig. 3, Ca.P.). The peduncles of the pre-anal papillae are larger than those of the post-anal. Spicules (Fig. 4, S.) are equal and 0·12 mm. in length. Gubernaculum is absent. 1551

Discussion.

The genus *Procamallanus* (Baylis, 1923) comprises nine species, *P. læviconchus* (Wedl, 1862), *P. spiralis* (Baylis, 1923), *P. parasiluri* (Fugita, 1927), *P. iheringi* (Travassos, 1928), *P. inopinatus* (Travassos, 1928), *P. rarus* (Travassos, 1928), *P. xenopodis* (Baylis, 1929), *P. mehrii* (Agrawal, 1930), and *P. sphæroconchus* (Tornquist, 1931). Of these only *P. mehrii* is hitherto recorded from India. These nine species fall into two groups :

those with ridges or leaf crowns in the inner part of the buccal capsule and those without them. The latter group includes only one species, *viz.*, *P. læviconchus* (Wedl, 1862). The species described in this paper resembles *P. læviconchus* in possessing a buccal capsule with smooth inner surface. It however differs from *P. læviconchus* in having equal spicules, seven pairs of pedunculated pre-anal papillæ and one pair of small post-anal papillæ. These differences are sufficient to justify creation of a new species for which I propose the name *Procamallanus planoralus* with the following specific diagnosis :

Procamallanus Baylis, 1923. Total length of male 3·85 mm., breadth 0·061 mm. ; of female 5·07 mm. and 0·079 mm. ; cuticle smooth and un-striated ; buccal capsule smooth, 0·082 mm. in length and 0·61 mm. in diameter ; two knob-like structures at the base of the buccal capsule ; œso-phagus 0·41 mm. in length ; nerve ring and excretory pore 0·24 and 0·31 mm. from the anterior end ; vulva about the middle of the body 1·4 to 3·38 mm. in front of the posterior end ; caudal alæ in male well developed ; seven pairs of pedunculated pre-anal papillæ and one pair of post-anal papillæ ; spicules equal 0·12 mm. in length, gubernaculum absent.

Host :—*Clarias batrachus* Bl.

Habitat :—Intestine.

Locality :—Nagpur, C.P., India.

EXPLANATION OF FIGURES.

Figs. 1–5.—*Procamallanus planoralus* n.sp.

Fig. 1. Anterior end. Dorsal view.

Fig. 2. Lateral view showing the position of female genitalia.

Fig. 3.—Posterior end of male showing caudal papillæ.

Fig. 4. Posterior end of male showing spicules.

Fig. 5.—Posterior end of female lateral view.

REFERENCE LETTERS.

A.	..	Anus.	*K.*	..	Knob-like structures
B.C.	..	Buccal capsule.	*L.*	..	Lobe.
C.A.	..	Caudal alæ.	*N.*	..	Nerve ring.
Ca.P.	..	Caudal papillæ.	*OE.*	..	Oesophagus.
G.A.	..	Genital aperture.	*P.L.*	..	Posterior lobe.
INT.	..	Intestine.	*S.*	..	Spicule.

R. B. Kulkarni

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STUDIES IN THE FAMILY *ALISMACEÆ.*

III. *Sagittaria guayanensis*[1] H.B.K. and *S. latifolia* Willd.

BY B. M. JOHRI,

.. *Department of Botany, Agra College, Agra.*

Introduction.

A REVIEW of the work on the family ALISMACEÆ has already been given in the first paper of the series (Johri, 1935). Of the genus *Sagittaria*, three species have so far been investigated. The earliest work is that of Schaffner (1897) on *S. variabilis*,[2] who found an eight-nucleate embryo sac with three antipodal cells. Cook (1907) also reported an eight-nucleate embryo sac with ephemeral antipodal cells in *S. lancifolia*. The fullest and the most recent account is that of Dahlgren (1931) on *S. sagittifolia*. This has a megaspore mother cell which undergoes the heterotypic division and produces two cells, of which the upper degenerates and the nucleus of the lower undergoes two divisions to produce a four-nucleate embryo sac. The two micropylar nuclei divide once again but the chalazal nuclei remain undivided, so that the mature embryo sac is six-nucleate. These findings agree with Dahlgren's earlier report on a few other members of the *Alismaceæ* (Dahlgren, 1928). I have also made some observations myself on *S. sagittifolia* (Johri, 1935), calling attention to the occasional occurrence of seven- and eight-nucleate embryo sacs in this plant.

Material and Methods.

The material of *S. guayanensis* was collected at Bharatpur in November 1933. Formalin-acetic-alcohol, Allen's modification of Bouin's fluid and Nawaschin's fluid were used for fixation. Of these, the last gave the best results. Flowers of *S. latifolia* were fixed in Nawaschin's fluid and very kindly sent by Dr. Norma E. Pfeiffer of The Boyce Thompson Institute, Yonkers, New York, in January 1934. The usual methods of infiltration and embedding were followed. Sections were cut 4-25 microns thick. The

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[1] A preliminary report of the work on *S. guayanensis* has already been published (Johri, 1934).

[2] This was really *S. latifolia* and will be referred to as such in the course of the paper. See Schaffner's later paper (1908), where the mistake is acknowledged.

slides were stained with Haidenhain's iron-alum hæmatoxylin and differenti-
ated in a saturated aqueous solution of picric acid. A very dilute solution
of fast green in alcohol was sometimes used as a counter-stain. A combina-
tion of crystal violet and erythrosin was also used with satisfactory results.

SAGITTARIA GUAYANENSIS.

Microsporogenesis.—The young anther is oval and consists of a mass
of meristematic cells. Later it becomes four-lobed, and simultaneously with
the appearance of these lobes there appears in each corner a group of hypo-
dermal archesporial cells which are distinguishable from the other cells by
their large nuclei and dense contents. The peripheral cells by periclinal
divisions cut off a primary parietal layer which divides to form two layers.
Of these the one adjacent to the epidermis is the endothecium, while the
inner divides again producing the middle layer and the tapetum (Fig. 1).
The endothecium develops the usual fibrous thickenings when the anther
is mature. The middle layer degenerates early and practically disappears
by the time the reduction divisions are over. The microspore mother cells
stay for a long time in the synizesis stage and round up soon after. The
walls swell and become mucilaginous. There are two successive divisions
and the arrangement of the microspores is isobilateral (Fig. 2).

Tapetum.—As in *Limnophyton* (Johri, 1935), the tapetal cells always
remain uni-nucleate. During the time the mother cells are undergoing
reduction, their walls become indistinct and the nuclei enlarge and stain
deeply. As soon as the microspores separate, the tapetal protoplasts become
amœboid and project into the anther lobe, their deeply staining nuclei soon
following. A little later the microspores are seen embedded in a mass of
periplasmodium with the tapetal nuclei scattered here and there (Fig. 3).
A certain amount of fusion of the protoplasts of individual tapetal cells may
sometimes occur even before their migration in between the microspores.
The periplasmodium is completely used up during the growth of the pollen
grains.

Tischler (1915) and Clausen (1927) have made similar observations in
several members of the Helobiales. The last author has described four types
of amœboid tapetum in monocotyledons :—

(i) THE SAGITTARIA-TYPE.—The tapetal cells lose their walls by the time
the tetrads are formed and their protoplasts begin to project inwards as
soon as the microspores have separated. Later the periplasmodium becomes
continuous. Examples : *S. sagittifolia*, *S. montevidensis*, *Alisma plantago*,
Limnocharis humboldti and *Hydrocharis morsus rance*. The same type is
seen in *Limnophyton obtusifolium* (Johri, 1935) and *S. guayanensis*.

(ii) The Butomus-type.—This type resembles the preceding in essential respects except that the periplasmodium formation occurs a little earlier when the microspores are still grouped into tetrads. Examples: *Butomus umbellatus, Stratiotes aloides* and *Ouvirandra* sp.

(iii) The Sparganium-type.—Here the chief point of difference is that the tapetal cells are multi-nucleate. Fusion of protoplast begins at the tetrad stage as in the Butomus-type. Examples: *Sparganium simplex, Typha latifolia, Tradescentia fluminensis* and *T. virginica.*

(iv) The Triglochin-type.—In this case the tapetum begins to show its activity while the microspore mother cells are still undergoing reduction. The tapetal protoplasts followed by their nuclei wander in between the
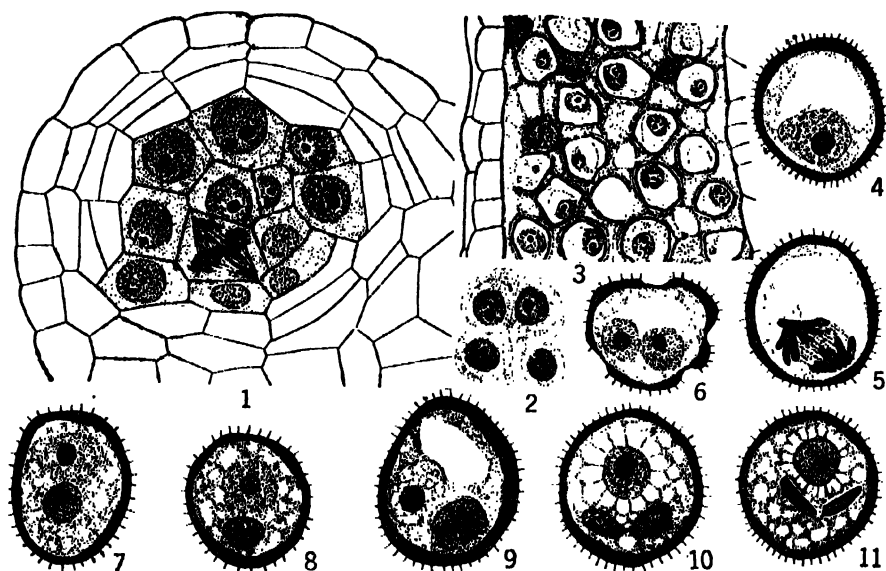
Figs. 1–11. Fig. 1.—T.S. anther lobe showing group of microspore mother cells, tapetum, a single middle layer and the endothecium. × 790. Fig. 2.—Isobilateral tetrad of microspores. × 790. Fig. 3.—A group of microspores surrounded by periplasmodium. × 395. Fig. 4. Uni-nucleate microspore. × 790. Fig. 5.—Division of the microspore nucleus. × 790. Figs. 6–8.—Pollen grains with tube and generative nuclei. × 790. Fig. 9. Same, the two nuclei separated by an ephemeral cell membrane. × 790. Fig. 10.—A tri-nucleate pollen grain with two spindle-shaped male nuclei. × 790. Fig. 11. The same, with two elongated male nuclei surrounded by a zone of light staining cytoplasm. × 790.

mother cells, so that the plasmodium is formed very early in comparison to the other three types. Examples: *Triglochin maritima, Potamogeton natans* and many Araceæ.

Male gametophyte. The centrally placed microspore nucleus moves near the wall. Here it is surrounded by dense cytoplasm while the rest of the pollen grain is occupied by a large vacuole (Fig. 4). The nucleus divides (Fig. 5) and produces two nuclei of approximately equal size with almost identical staining capacity (Fig. 6). One of these increases in size (Fig. 7), while the other does not increase in size but becomes richer in chromatin and takes a deeper stain (Fig. 8); the former is the tube nucleus while the latter is the generative nucleus. Sometimes an ephemeral cell plate is laid down between these two nuclei (Fig. 9), but due to its early disorganisation they soon lie free in the cytoplasm. This agrees with the earlier observations of Schürhoff (1926) on *S. sagittifolia.*

The generative nucleus divides inside the pollen grain and produces two spherical male nuclei which soon become spindle-shaped (Figs. 10 and 11). Usually there is no distinction between the general cytoplasm of the pollen grain and that surrounding the male nuclei; but occasionally a very delicate zone of lighter staining cytoplasm was distinguishable round the male nuclei (Fig. 11) and both kinds of pollen grains may be seen in the same anther. Tri-nucleate pollen grains have also been reported in the family *Alismaceæ* in *S. latifolia* (Schaffner, 1897), *S. sagittifolia* (Schürhoff, 1926), *Elisma natans* (Dahlgren, 1928) and *Limnophyton obtusifolium* (Johri, 1935). In the last named plant Narasimha Murthy (1933) reports that the male nuclei have a definite sheath of cytoplasm around them and are therefore *male cells.*

*The ovule.—*The ovules are anatropous (Fig. 12). The two integuments are each two cells thick, but the part forming the micropyle is usually
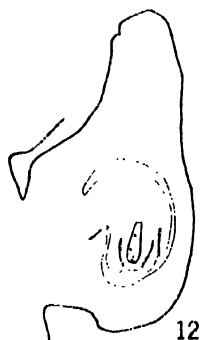
Fig. 12. L.S. carpel showing an anatropous ovule with a four-nucleate embryo sac ✕ 80.

thicker. The inner integument appears about the time, when the megaspore mother cell is in the synizesis stage, and is fairly well advanced by the time it is undergoing reduction (Fig. 17). The outer grows more slowly and in later stages it fuses with the adjacent cells of the inner integument.

In one case I came across a pair of nucelli enclosed within the same two integuments. One embryo sac was present in each, but the exact stage of development could not be determined as the preparation was not satisfactory. Dahlgren (1928) has also figured a similar case in *Damasonium alisma* with each nucellus containing a megaspore mother cell.

The archesporium.—The archesporium is differentiated in the nucellus long before the carpel has closed. Usually there is a single hypodermal archesporial cell (Fig. 13), but sometimes there may be two cells lying side by side (Fig. 14) or one upon the other (Fig. 15). Fig. 16 gives an indication of the presence of three archesporial cells lying side by side. Holmgren (1913) found a many-celled archesporium in *Butomus umbellatus*, a plant belonging to the closely allied family Butomaceæ.
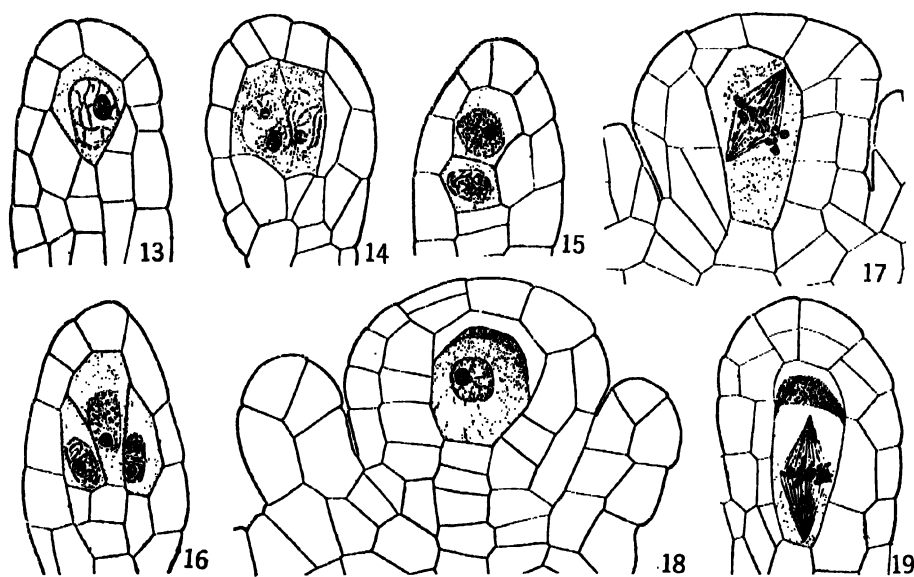
Figs. 13–19. × 630. Fig. 13. Young nucellus showing hypodermal archesporial cell. Fig. 14. —Nucellus with two archesporial cells lying side by side. Fig. 15. Same with two archesporial cells lying one upon the other. Fig. 16. Three archesporial cells. Fig. 17. Megaspore mother cell in metaphase. Fig. 18. Daughter cells formed after heterotypic division; upper degenerating. Fig. 19. Nucleus of the lower dyad cell in metaphase.

The archesporial cell functions directly as the megaspore mother cell without cutting off a wall cell. Similar observations have also been made in *Alisma plantago, Damasonium alisma, Echinodorus ranunculoides* (Dahlgren, 1928); *Limnophyton obtusifolium* (Johri, 1935; Narasimha Murthy, 1933);

Sagittaria sagittifolia (Dahlgren, 1934 ; Johri, 1935) and *Echinodorus macro-phyllus* (Dahlgren, 1931). Fig. 17 shows a megaspore mother cell in the metaphase of the heterotypic division. On division it produces two cells of which the outer is much smaller (Fig. 18). The nucleus of this cell stains rather deeply and the cytoplasm assumes a crescent-shaped appearance. It begins to disorganise very early but its degenerated remains may some-times be detected on the top of the embryo sac. It is this cell which Schaffner (1896) mistook for a wall cell in *Alisma plantago* and judging from his figures, Hall (1902) appears to have done the same for *Limnocharis emarginata*. Rarely the nucleus of the upper dyad cell may divide once (Fig. 21), but sometimes the degeneration is so quick that the division starts but is not completed.

The development of the embryo sac.—A tetrad of megaspores is not pro-duced. The lower dyad cell grows into the embryo sac and thus the deve-lopment is of the "Scilla-type". Fig. 19 shows the nucleus of this cell in metaphase. Fig. 20 represents the next stage, in which the nucleus has divided into two and the cell has elongated considerably. The two nuclei now move towards the poles and a vacuole appears between them (Fig. 21). It is to be noted that the primary chalazal nucleus is from the very beginning appreciably smaller than the primary micropylar nucleus. Each of the two nuclei now undergoes a further division (Fig. 22), and four nuclei are formed, of which two lie at each pole of the embryo sac (Fig. 23). About this stage or even earlier the nucellar epidermis becomes two-layered at several points by periclinal divisions.

The two micropylar nuclei divide once again but the two chalazal nuclei as a rule do not divide further and the embryo sac thus remains only six-nucleate. Ephemeral cell plates sometimes appear on the spindles (Fig. 24). In this particular case figured here, it is quite possible that the primary chalazal nucleus of the two-nucleate stage may not have divided when the micropylar nucleus divided into two, and then all the three nuclei (two at the micropylar end and one at the chalazal end) divided simultaneously resulting in six daughter nuclei.

The four micropylar nuclei give rise to the egg apparatus and the upper polar nucleus. From the lower two, arise the lower polar nucleus and the single antipodal. The synergids are pear-shaped and occasionally hooked with a large vacuole in the broad basal part and the nucleus in the upper part just below the filiform apparatus (Fig. 26). One synergid persists for a considerably long time, even after fertilisation (Figs. 28–32). The egg nucleus always lies in the lower part of the egg which protrudes below the synergids. The upper polar nucleus, which is the largest of all the nuclei
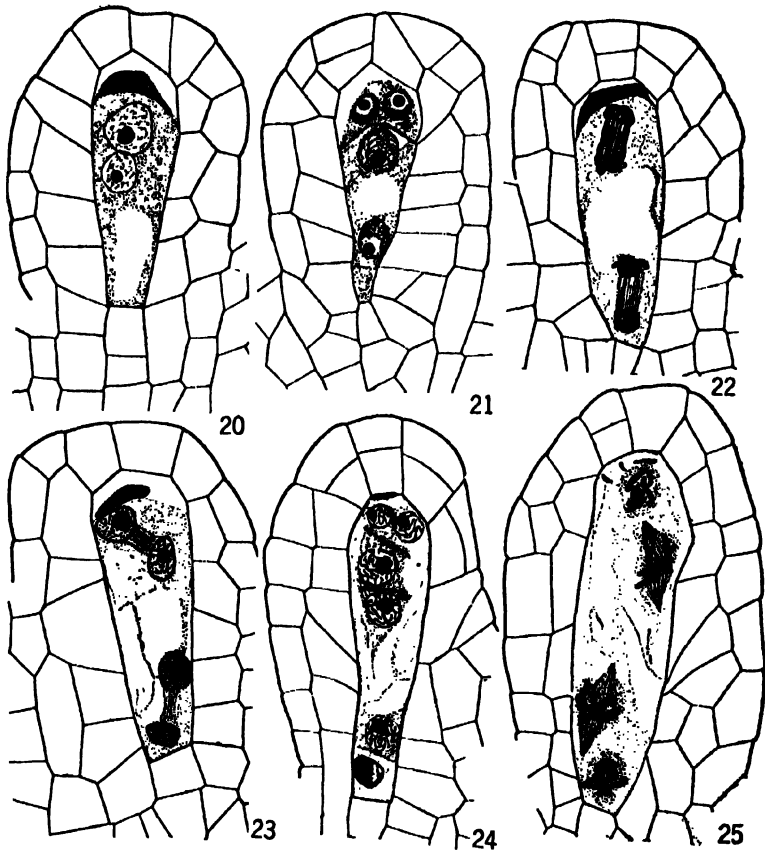
Figs. 20-25. ✕ 630. Fig. 20.— Two-nucleate embryo sac with degenerated cell above. Fig. 21.—Two-nucleate embryo sac with the nucleus of the upper dyad cell divided into two. Fig. 22.—Division of two nuclei into four. Fig. 23.—Four-nucleate embryo sac. Fig. 24. Six-nucleate embryo sac with two micropylar spindles and one chalazal spindle in late telophase; cell plates are visible. Fig. 25.— All the four nuclei of a four-nucleate embryo sac are dividing.

in the embryo sac, lies very close to the egg. The lower polar nucleus and the single antipodal lie in the narrow chalazal end. The two polar nuclei meet in the middle of the embryo sac, but actual fusion is delayed till the arrival of the male nucleus.

This describes the usual course of events. But it is important to note that occasionally one or both the chalazal nuclei of the four-nucleate stage may divide once, producing seven- or eight-nucleate embryo sacs respectively (Figs. 27, 29). Fig. 25 is drawn from a lucky preparation in which all four nuclei show metaphasic spindles. There are also indications that sometimes

the number of the antipodal nuclei may increase by fragmentation of the lowest chalazal nucleus of the tetra-nucleate stage. Similar cases were seen by me in *Limnophyton* (Johri, 1935) and *S. sagittifolia* (Johri, 1935). In all cases the antipodal nuclei took a dark stain with hæmatoxylin. I never saw them separated by distinct walls, though ephemeral membranes are occasionally present (Fig. 26).
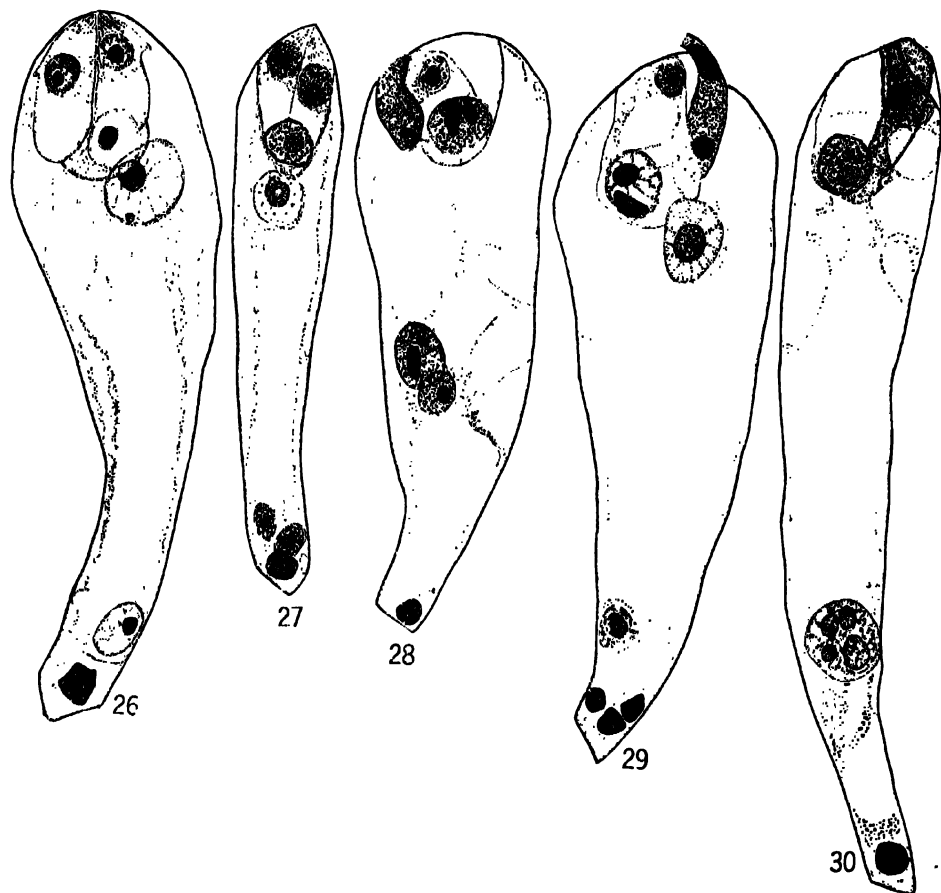
FIGS. 26 30. × 630. Fig. 26.—Six-nucleate embryo-sac. An ephemeral membrane is seen separating the lower polar nucleus from the single antipodal nucleus. Fig. 27. · Seven-nucleate embryo sac. Fig. 28.- Stage in double fertilisation. One male nucleus appressed to the egg nucleus and the other to the polar nuclei. Fig. 29. –Belated fertilisation of the egg. Fig. 30.—Fertilised embryo sac.

Pollination and Fertilisation.

Several whorls of creamy white flowers usually with three flowers in each whorl are arranged on the scape. In the lower whorls the flowers are

mostly bisporangiate, while those in the upper whorls are chiefly staminate with rudimentary carpels in the centre. The ovules in the rudimentary carpels have been seen to develop as far as the megaspore mother cell stage in *S. montevidensis* (Sykes, 1909). In *S. guayanensis* the megaspore mother cell goes through the heterotypic division, but I have not observed any further development after the dyad stage in the carpels of the staminate flowers. In *S. sagittifolia* (Johri, 1935) the development progresses up to the formation of a four-nucleate embryo sac but at this stage perhaps the pollen is shed and the flowers wither and fall off.

Well-developed nectaries are present and pollination occurs through the agency of insects. In hermaphrodite flowers the stamens mature much earlier than the carpels, thus minimising the chances of self-pollination.

During its passage through the narrow micropyle, the pollen tube is very thin and often difficult to trace, but as soon as it enters the embryo sac it swells up. It always disorganises one of the synergids and may push the egg to one side. Of the two male nuclei, easily distinguishable by their spindle-shaped outline and high staining capacity, one fuses with the two polar nuclei and the other with the egg. Triple fusion either occurs simultaneously with the fertilisation of the egg or even before it (Fig. 28). I saw only one case in which the egg had already been fertilised, while the polar nuclei were at their original positions and the second male nucleus still in the pollen tube (Fig. 29). The tube nucleus was seen to remain behind and degenerate within the pollen tube (Fig. 30). Double fertilisation has so far been reported in the following plants of the family *Alismaceæ* :—*Alisma plantago* (Nitzschke, 1914 ; Dahlgren, 1928) ; *S. latifolia* (Schaffner, 1897) ; *Limnophyton obtusifolium* (Narasimha Murthy, 1933) and *Echinodorus macrophyllus* (Dahlgren, 1931). To these may now be added *Sagittaria guayanensis.*

Endosperm.

The primary endosperm nucleus always divides prior to the division of the egg. The two daughter nuclei are separated by a wall, which divides the embryo sac into a large micropylar and a small chalazal chamber (Fig. 31). Schaffner (1897) made a similar observation in *S. latifolia* and Cook (1907) in *S. lancifolia*. In *Limnophyton* (Johri, 1935) a distinct wall is not formed but an ephemeral cell plate is present. In *Alisma plantago, Damasonium alisma* and *Elisma natans* the endosperm is of the nuclear type (Dahlgren, 1928) ; while in *S. sagittifolia* and *Echinodorus macrophyllus* it is of the typical helobiales type (Dahlgren, 1931).

The nucleus of the micropylar chamber moves upwards and undergoes many free nuclear divisions. The nucleus of the chalazal chamber is much
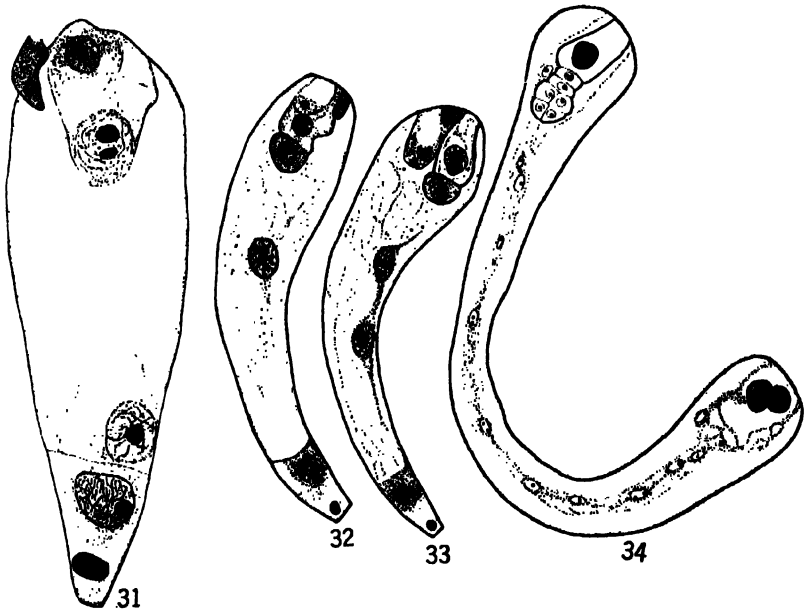
FIGS. 31–34. Fig. 31. First division of the primary endosperm nucleus; daughter nuclei separated by a cell wall. × 630. Fig. 32. Fertilised egg divided into two cells; the endosperm nucleus of the micropylar chamber has migrated to the middle. × 284. Fig. 33.—The same with two endosperm nuclei in the micropylar chamber; the nucleus of the chalazal chamber showing signs of division. × 284. Fig. 34. Numerous free nuclei lying in the micropylar chamber and two nuclei in the chalazal chamber. × 175.

less active; usually it divides only once and the two nuclei degenerate (Figs. 32–34). Rarely there may be 3 or 4 nuclei formed as a result of further division. The embryo sac has also been growing actively during this time and becomes almost doubled up on itself. At one extremity there is the developing embryo and at the other the degenerating endosperm nuclei. The endosperm is gradually used up, as all the available space of the embryo sac is occupied by the embryo.

Embryo.

The first division of the oospore is transverse dividing the egg into a large basal and a small terminal embryo cell (Fig. 35). The basal cell does not divide further and the subsequent growth of the embryo depends on the activity of the terminal cell which divides transversely (Fig. 36). The next division occurs in the middle cell and a proembryo of four cells is formed (Fig. 37). Further divisions are irregular. In Fig. 38 the terminal cell has divided first by a vertical wall, while Fig. 39 shows the division of the cell
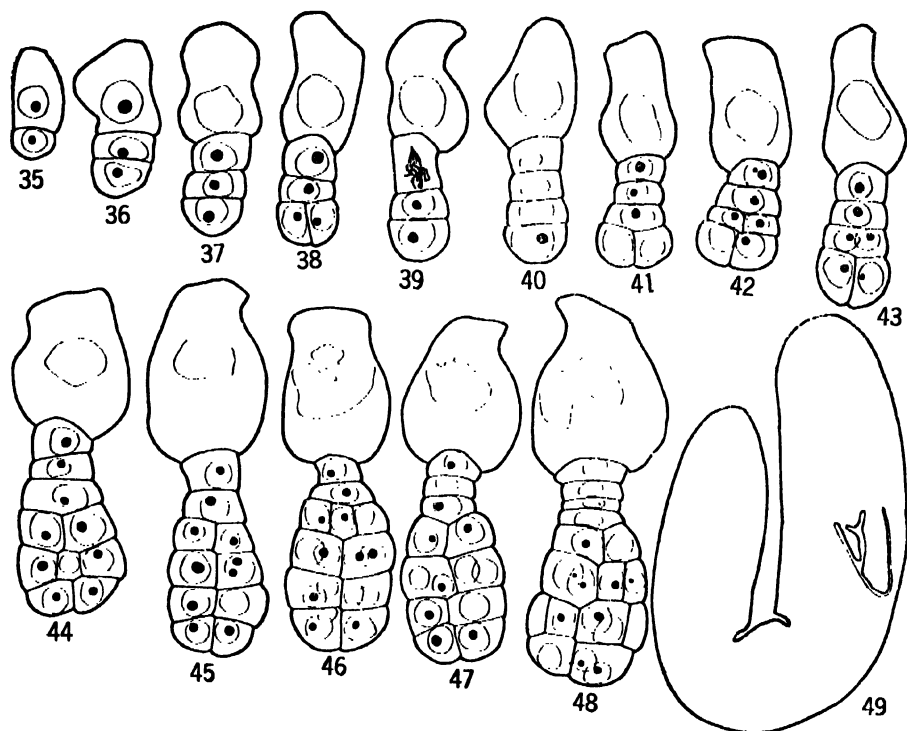
Figs. 35 49. ✕ 361. Development of the embryo. For explanation see text.

adjacent to the basal cell, finally producing a row of five cells (Fig. 40). Fig. 41 shows the division of the terminal cell of a five-celled proembryo by a vertical wall. Figs. 42–43 show the division of the cell adjacent to the terminal cell. Further development illustrated by Figs. 44–48, is essentially in accordance with the observations of other authors (Schaffner, 1897; Souèges, 1931). Fig. 49 shows a fully formed horse-shoe shaped embryo in which all the parts have differentiated.

SAGITTARIA LATIFOLIA.

Since most of the plants of the family *Alismaceæ* that have been so far investigated, show a six-nucleate embryo sac, I was tempted to reinvestigate *S. latifolia*, which is the only member of this family reported to have an eight-nucleate embryo sac (Schaffner, 1897). The material sent by Dr. N. Pfeiffer for this investigation was collected late in the season and therefore earlier stages leading to the development of the gametophytes could not be found. A periplasmodium is formed as in other members of the family.

Male gametophyte.—In two-nucleate pollen grains the tube and generative nuclei are separated by an ephemeral membrane (Fig. 50) but this
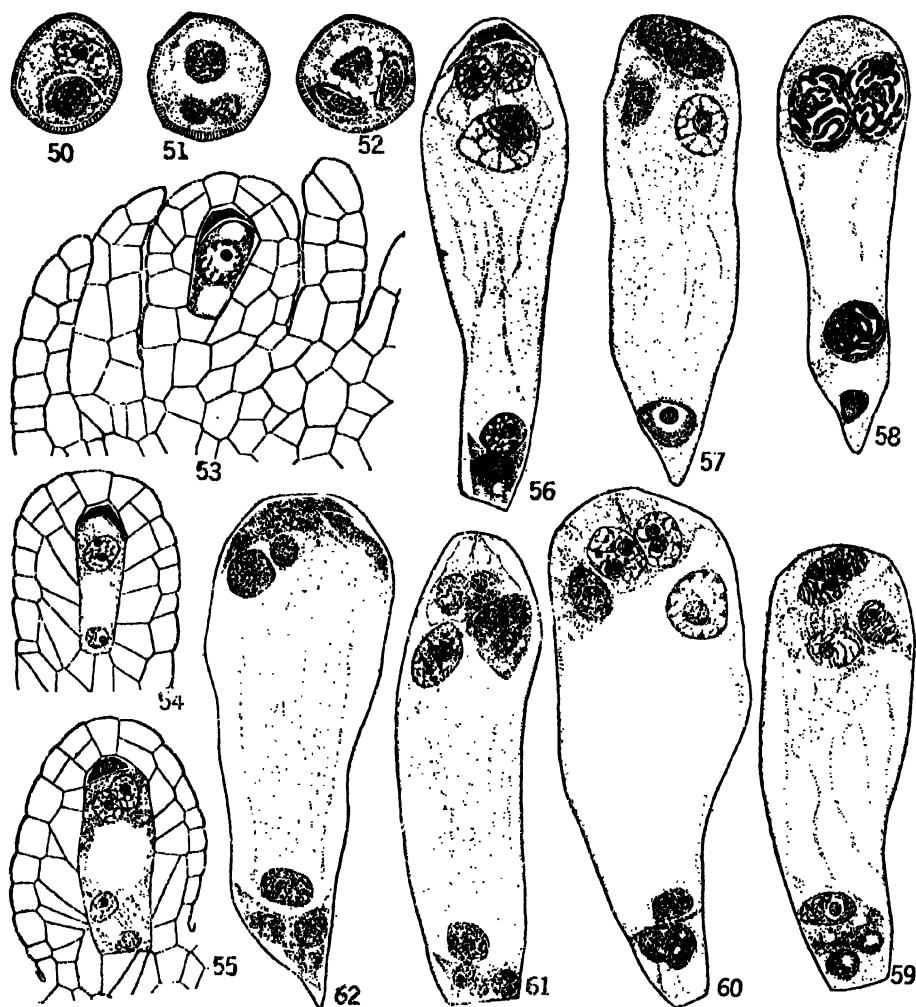
FIG. S. 50-62.—*Sagittaria latifolia*. Fig. 50.—A two-nucleate pollen grain with tube and generative nuclei separated by an ephemeral cell membrane. Fig. 51.—Three-nucleate pollen grain with spherical male nuclei. Fig. 52. Same with spindle-shaped male cells. Fig. 53. Daughter cell formed after the heterotypic division of the megaspore mother cell; upper cell degenerating and the lower enlarging. Fig. 54. Two-nucleate embryo sac with degenerated cell above. Fig. 55. Four nucleate embryo sac with remains of the degenerated cell. Nucellar epidermis dividing. Fig. 56. Six-nucleate embryo sac with remains of the degenerated dyad cell, whose nucleus occasionally divides before the cell degenerates. Fig. 57. Five-nucleate embryo sac. The primary chalazal nucleus has entirely failed to divide. Fig. 58. Three nuclei of a four-nucleate embryo sac undergoing division. Fig. 59. Seven nucleate embryo sac; the two antipodal nuclei probably formed by the division of the lowest chalazal nucleus of the four-nucleate stage. Fig. 60. Same, the chalazal nucleus showing signs of fragmentation. Fig. 61. Eight-nucleate embryo sac. All the three antipodal nuclei are lying in a common cytoplasmic mass. The egg is situated laterally as in Figs. 59 and 60. Fig. 62. Same with well-defined antipodal cells. The egg-apparatus has degenerated. Figs. 50-52 and 56-62 × 750. Figs. 53-55 × 370.

seems to have been overlooked by Schaffner (1897) due to its quick disappearance. The generative nucleus divides and produces two spherical male nuclei (Fig. 51). According to Schaffner the pollen is shed in this condition and the male nuclei become spindle-shaped only afterwards. I have seen pollen grains with spherical as well as spindle-shaped male nuclei (Fig. 52) in the same anther loculus. Schaffner was unable to decide whether definite sperm cells are organised or not. In my preparations I have often seen the sperm nuclei invested with a very delicate lightly staining area. He further says that when the sperm nuclei have become spindle-shaped the tube nucleus takes a very dark stain (probably with anilin-safranin and gentian violet; see p. 252). I find that with haematoxylin it stains very lightly and sometimes remains yellowish.

Female gametophyte.—The earlier stages were not seen by Schaffner. The earliest stage, I could obtain, is shown in Fig. 53. Although a hypodermal megaspore mother cell was not seen in the material at my disposal, I have no doubt that this does occur and the two cells shown in this figure are formed from its division. The upper degenerates and the lower produces the embryo sac (Fig. 53). The nucleus of this cell divides to produce the primary micropylar and the primary chalazal nuclei of which the latter is smaller (Fig. 54). Each of the nuclei now divides once again and a four-nucleate embryo sac is formed (Fig. 55). Schaffner states that all the four nuclei divide once and produce an eight-nucleate embryo sac.

Here my observations differ from Schaffner's, for I find that in the majority of cases the two chalazal nuclei of the four-nucleate embryo sac do not undergo any further division and thus a six-nucleate embryo sac is produced (Fig. 56) as in other members of the family that have been recently investigated by Dahlgren and myself. Rarely the primary chalazal nucleus may entirely fail to divide so that the embryo sac may remain only five-nucleate (Fig. 57)—a condition also seen in *Echinodorus* (Dahlgren, 1928, 1931).

It is true that *sometimes* seven- and eight-nucleate embryo sacs are also formed (Figs. 58–62). In Fig. 58 only three nuclei—two micropylar and one chalazal—are dividing and this would result in the formation of a seven-nucleate embryo sac. Figs. 59 and 60 show such embryo sacs. Less frequently both the chalazal nuclei (of the four-nucleate stage) may divide resulting in eight-nucleate embryo sacs (Figs. 61–62).

The synergids are hooked and the egg may be sometimes placed laterally. The upper polar nucleus is the largest in size. The antipodal nuclei can organise into cells (Fig. 62), but sometimes two or even all the three nuclei may be enclosed in a common cytoplasmic mass (Figs. 56, 59–61).

Summary.

SAGITTARIA GUAYANENSIS.

1. The anther has four groups of archesporial cells. The primary parietal layer produces the endothecium, a single middle layer and the tapetum. The middle layer degenerates very early and the tapetum produces the periplasmodium which is used up as the pollen grains mature.

2. The microspore mother cells undergo two successive divisions and produce isobilateral tetrads of microspores. The microspore nucleus divides to produce the tube and generative nuclei. The generative nucleus divides again and produces two spindle-shaped male nuclei. The mature pollen grain is tri-nucleate.

3. The ovules are anatropous with two integuments. The embryo sac becomes curved in later stages.

4. There is usually a single hypodermal archesporial cell in the nucellus. This functions directly as the megaspore mother cell. It divides into two cells of which the upper degenerates and the lower produces the embryo sac.

5. The development proceeds in the normal way up to the four-nucleate stage. The two micropylar nuclei now divide further to produce the egg-apparatus and the upper polar nucleus. The chalazal nuclei usually do not divide further ; of these the upper functions as a polar nucleus while the remaining nucleus is the only antipodal.

6. Sometimes one or both of the chalazal nuclei divide and the mature embryo sac becomes seven- or eight-nucleate.

7. Double fertilisation occurs. Syngamy occurs about the same time as the triple fusion or a little later.

8. After the first division of the primary endosperm nucleus the two daughter nuclei are separated by a wall, which divides the embryo sac into a large micropylar and a small chalazal chamber. The endosperm is formed by free nuclear divisions in the micropylar chamber. The nucleus of the chalazal chamber divides once or twice and then the daughter nuclei degenerate.

9. The development of the embryo corresponds to the Alisma type.

S. LATIFOLIA.

10. A reinvestigation of *S. latifolia* shows that this plant is very similar to the other members of the *Alismaceæ* in having a six-nucleate embryo sac and that the seven- and eight-nucleate conditions are much less frequent.

I am greatly indebted to Dr. P. Maheshwari for his constant help, guidance and interest in my work. I am also thankful to Dr. Norma E. Pfeiffer without whose help the work on *S. latifolia* could not have been possible. To the authorities of the Agra University I am grateful for financial help during the course of this work.

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THE LIFE-HISTORY OF *TRIANTHEMA MONOGYNA* LINN.

BY H. R. BHARGAVA,

Department of Botany, Agra College, Agra.

Introduction.

A SEARCH through the available literature shows that little attention has been paid to the family Aizoaceæ and whatever embryological work has been done on this family has already been reviewed in my previous paper on *Mollugo nudicaulis* (Bhargava, 1934).

The material used for this investigation was collected locally and fixed in formalin-acetic-alcohol, corrosive sublimate-formalin-acetic-acid-alcohol and chrom-acetic acid (chromic acid 1 gm., acetic acid 3 c.c. and water 100 c.c.). For older stages in the development of the embryo, the ovules were dissected out before fixing. As even these did not cut satisfactorily, some ovules were again fixed in formalin-acetic-alcohol, and imbedded in paraffin after employing the Butyl alcohol method recommended by Zirkle (1930). The results obtained by this method were much more satisfactory. Sections were cut 5-15 microns thick, stained with Iron-alum Hæmatoxylin and differentiated in an aqueous solution of picric acid (Maheshwari, 1933).

Investigation.

Microsporogenesis.— The earliest stage that I found in a transverse section of the anther shows the primary sporogenous cell with a parietal cell cut off on the outside (Fig. 1). The latter by further anticlinal and periclinal divisions gives rise to four wall layers—the endothecium, two middle layers and the tapetum (Figs. 2-4). The tapetal cells are at first uni-nucleate (Figs. 2 and 3) but at the time when the microspore mother cells are in synizesis, the nuclei divide mitotically and henceforward they are bi-nucleate (Fig. 4). The endothecium develops the usual fibrous thickenings at maturity.

In the meantime the primary sporogenous cells divide to form a number of microspore mother cells which have prominent nuclei and are filled with dense cytoplasm. In a cross-section of the anther two to three microspore mother cells are seen in each lobe, and each row extends five to nine cells deep in a longitudinal section. As in *Lathræa clandestina* (Gates, 1924) the

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pollen mother cells do not round off and separate from each other, but remain in contact throughout the whole process of meiosis until the final dissolution of their walls. The original cell walls thus retain their polyhedral shape for a long time, while a special mother cell wall, of a different composition and often of great thickness, is laid down inside and in contact with the mother cell wall. When contraction occurs in fixing, the special wall often remains attached to the mother cell wall while the cytoplasm contracts away from it (Figs. 6-8). As soon as the first reduction division is over, the two daughter nuclei enter the second reduction division without any wall being laid down
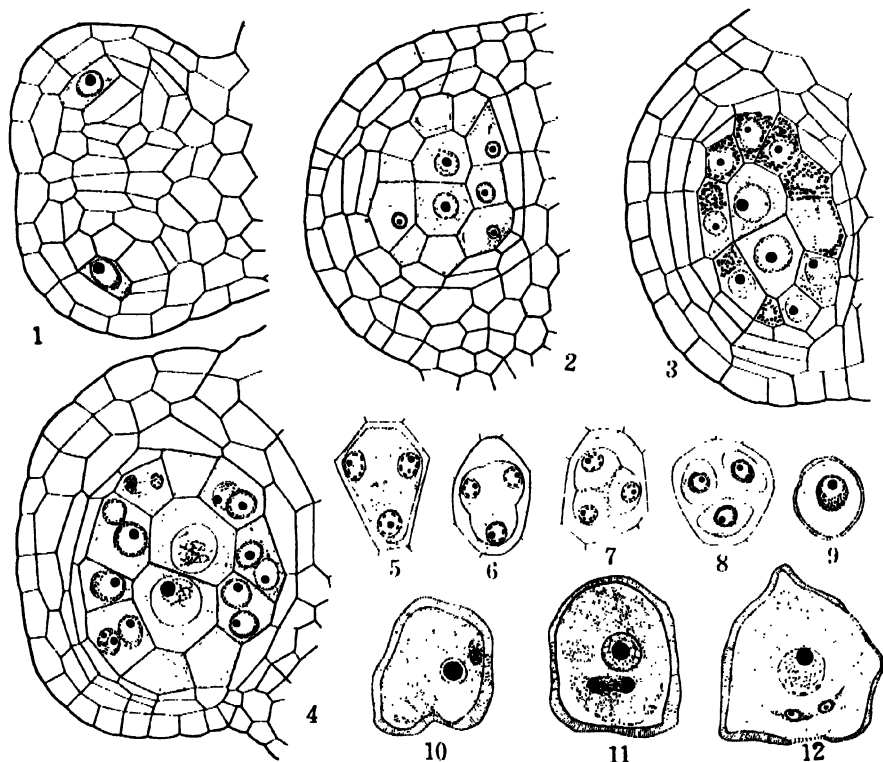
Figs 1-4.—Parts of transverse sections of anthers. × 640. Fig. 1.—In one lobe the archesporial cell has divided to form the primary wall cell and primary sporogenous cell; in the other lobe the primary wall cell has also divided once by an anticlinal wall. Fig. 2.—Two microspore mother cells surrounded by the uninucleate tapetum, 2-3 wall layers and the epidermis. Fig. 3.—Older stage showing four wall layers including the tapetum. Fig. 4.—Mother cells in synizesis and the tapetal cells are two-nucleate.

Figs. 5-8.—Stages in the development of the microspores showing quadripartition by furrowing; the special mother cell wall is shown by light dots. × 640.

Figs. 9-12.—Stages in the development of the male gametophyte. × 640.

between them. After the reduction divisions are over the first evidence of
further development in the pollen mother cells is the beginning of constriction
furrows in the cytoplasm at four points placed at equal intervals on its peri-
phery (Figs. 5–7). These become progressively deeper until they meet in
the centre and finally cut up the cytoplasm into four masses. At this stage
the heavy special mother cell wall is seen to be entering in between the
microspores and separating them from one another (Fig. 8). Soon after the
original mother cell wall and then the special mother cell wall rapidly dissolve,
setting free the four microspores. The development and behaviour of a
similar special mother cell wall has been observed by Gates (1924) in *Lathræa
clandestina*. The arrangement of the microspores is tetrahedral (Fig. 8).

Slight differences were observed in the relative development attained
by the anthers of the same flower, as in some cases one or two stamens already
had young microspores, while in others the development has not proceeded
beyond synizesis in the microspore mother cells. Instances were also seen
where, in the same anther, the microspore mother cells had completely
degenerated in one lobe while in the other three lobes they had a perfectly
normal appearance. The usual intine and exine get differentiated after the
microspores have separated, rounded up and enlarged (Figs. 9–12). The
nucleus of the microspore divides to form a large tube cell and a small crescent-
shaped generative cell (Fig. 10). Later, the nucleus of the generative cell
divides (Fig. 11) giving rise to two male cells, which are more or less spindle-
shaped (Fig. 12). There are three germ pores on each pollen grain (Fig. 9).

Ovule.—There are about eight to nine ovules in the ovary. The ovules
become amphitropous at a very early stage of their development (Fig. 13).
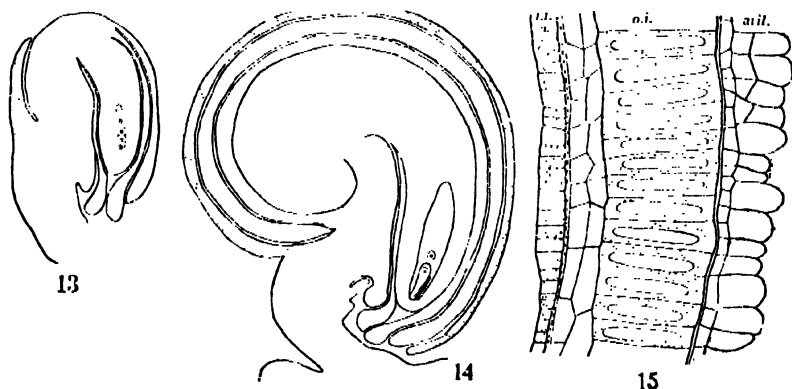
Figs. 13–14.—Longitudinal sections of ovules showing the development of the aril. $\times$ 95.
Fig. 15.—Longitudinal section of seed, showing a part of the seed coat. *i.i.*, Inner integu-
ment; *o.i.*, Outer integument. $\times$ 95.

As in other members of the family, the inner integument is only two cells thick except at the end forming the micropyle where it is thicker. Unlike any other member of the family so far investigated the outer integument is three-layered, and further an aril or third integument begins to develop as early as the megaspore mother cell stage. At the megaspore tetrad stage it is sufficiently conspicuous (Fig. 13) and by the time of fertilisation it almost completely surrounds the ovule (Fig. 14). So far I am aware this is the only plant of the family which has been found to have an aril.

Megasporogenesis.—Usually there is a single hypodermal archesporial cell (Fig. 16) which divides periclinally into a primary wall cell and the megaspore mother cell (Fig. 17), but judging from the condition shown in Fig. 20 it seems that occasionally no wall cell is cut off and the archesporial cell functions directly as a megaspore mother cell. Sometimes two mother cells have also been found (Fig. 18) and the fact, that in one case a two-nucleate embryo sac was seen together with an eight-nucleate embryo sac in the same nucellus, seems to indicate that occasionally both may function. It is at the megaspore mother cell stage that the two integuments make their appearance and as mentioned earlier the aril or the third integument appears soon after. The megaspore mother cell enlarges and elongates considerably (Fig. 19), and after the first reduction division is over a wall is laid down between the daughter nuclei (Fig. 20), the lower of the two daughter cells being frequently larger than the upper. The second reduction division now follows (Fig. 21), but the mitosis in the two dyad cells may not proceed at the same rate. The lagging behind of the division in the upper cell (Figs. 21, 22) and the absence of a cell plate, even when the nucleus has divided (Fig. 23), may be taken as evidences of a tendency towards the formation of a row of three cells instead of four. A similar condition has been recently reported by Mauritzon (1934) in some plants of the allied family Phytolaccaceæ—*Rivinia humilis, R. brasiliensis, Phytolacca octandra, Petiveria alliacea* and *Villamilla peruviana.*

The primary wall cell usually does not undergo any periclinal divisions but the cells of the epidermis divide freely as can be seen from Figs. 19-25, and a five to six-layered tissue is formed. There are, however, about three to six epidermal cells, lying just above the embryo sac, which do not divide but simply elongate radially (Figs. 22-25) as in *Mesembrianthemum pseudotruncatellum* (Schmid, 1925), *M. linguiforme* and *Tetragonia expansa* (Huber, 1924).

The chalazal megaspore enlarges and functions, while the other two degenerate. The two-, four- and eight-nucleate stages of the embryo sac are passed through in the normal way (Figs. 24-26). At the four-nucleate stage the cells of the nucellus adjacent to the embryo sac begin to disinte-
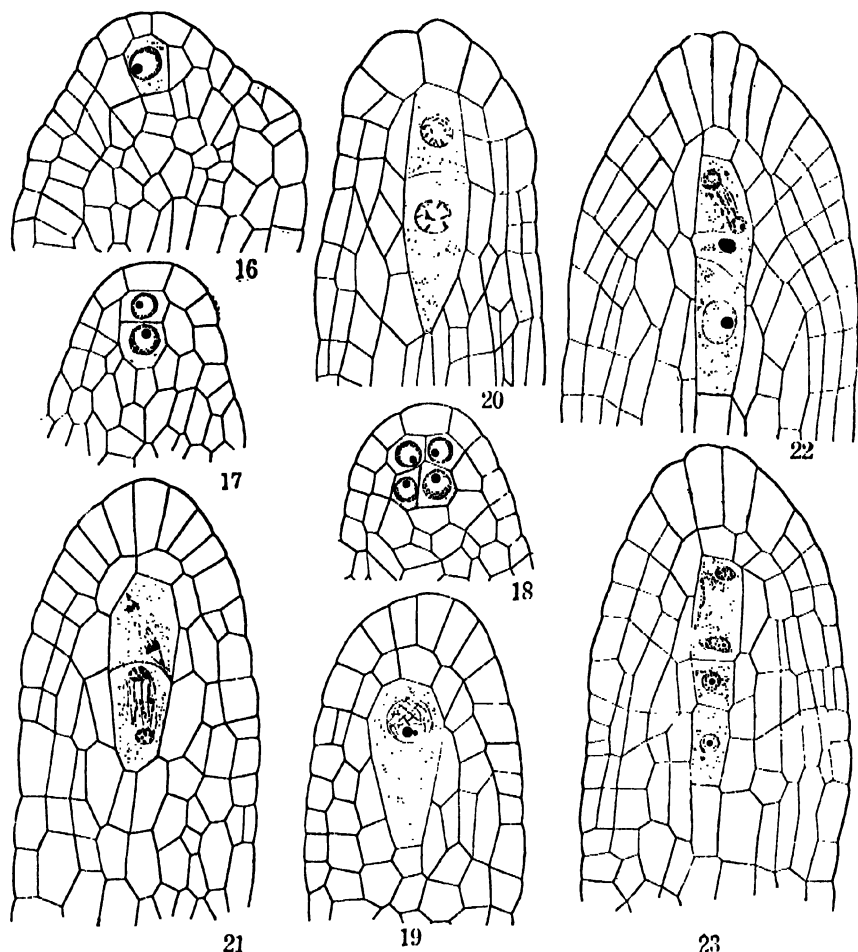
Figs. 16–23.— Development of megaspores. × 640. Fig. 16.—Hypodermal archesporial cell. Fig. 17.—Primary wall cell and megaspore mother cell. Fig. 18. Two megaspore mother cells and two wall cells. Fig. 19.—An enlarged and elongated mother cell. Fig. 20. A dyad of megaspores; no wall cell has been cut off here. Fig. 21.—The two dyad cells dividing. Fig. 22.— Three megaspores, the chalazal megaspore has enlarged, the middle one is on its way to degenerate, and in the micropylar cell the nuclear division is not yet over. Fig. 23.—Same, the micropylar cell has two megaspore nuclei unseparated by a wall.

grate and degenerate (Fig. 25) and the mature embryo sac comes to lie immediately below the radially stretched cells of the nucellar epidermis. The polar nuclei fuse long before fertilisation. The antipodal cells are ephemeral and disappear at the time of fertilisation. At this stage the chalazal end of the embryo sac begins to push downwards into the tissue of the nucellus.
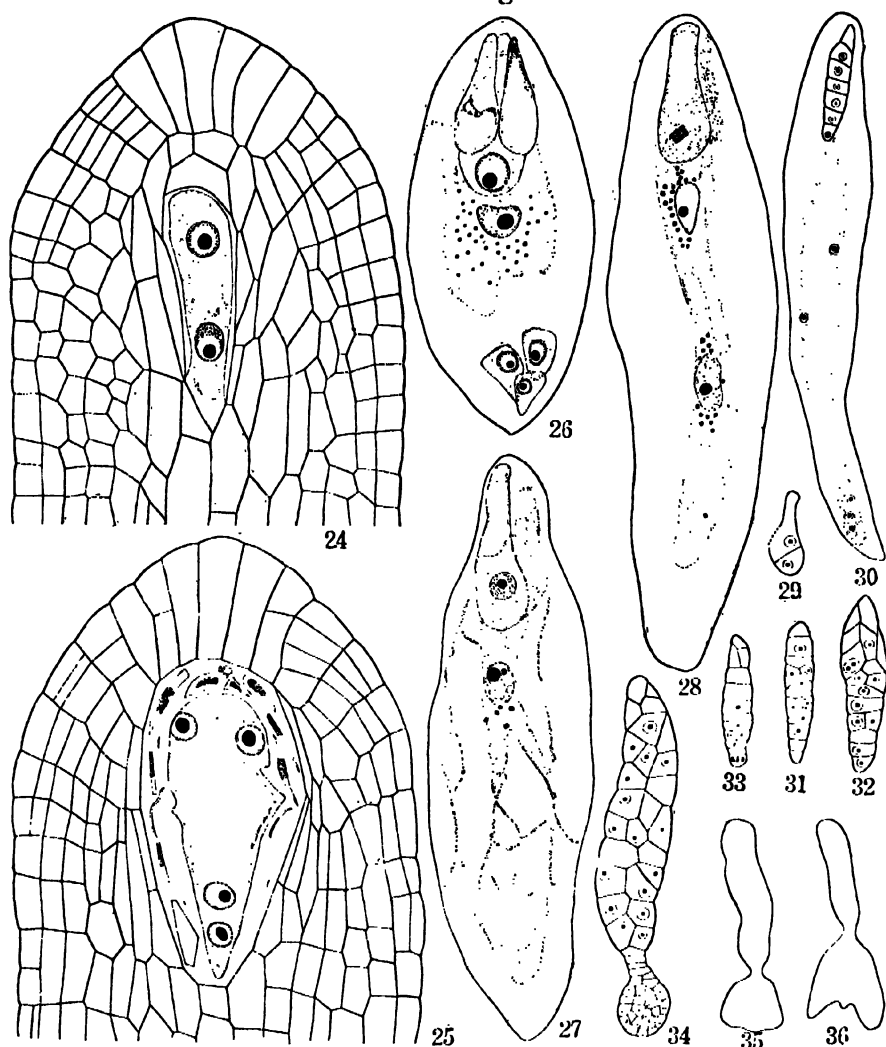
FIGS. 24-27.—Stages in the development of the embryo sac. × 395. Fig. 24.—Longitudinal section of nucellus with a two nucleate embryo sac; the epidermal cells above the embryo sac have elongated radially. Fig. 25. Same, with four-nucleate embryo sac. Fig. 26.—Mature embryo sac; the black dots round the fusion nucleus represent the starch grains. Fig. 27. —Older embryo sac having the fertilised egg and the primary endosperm nucleus; the remains of the pollen tube can be seen on the right-hand side of the egg.

FIG. 28.— More advanced stage with two endosperm nuclei and the fertilised egg dividing. × 395.

FIGS. 29-36.—Stages in the development of the embryo. Fig. 29.—A two-celled embryo. Fig. 30.—An embryo-sac with a filamentous pro-embryo and the free nuclear endosperm. Figs. 31-32.—Further stages in the development of the embryo. Fig. 33. —A young pro-embryo with the terminal cell dividing. Fig. 34.—A globular embryo with a long and massive suspensor consisting of several cell rows. × 170. Fig. 35.—An older embryo with the two cotyledons marked out. Fig. 36.— More advanced stage with cotyledons and stem tip clearly distinguishable. × 63.

The mature embryo sac of *Trianthema monogyna* unlike that of *Mollugo nudicaulis* (Bhargava, 1934) has been found to contain starch grains scattered round the fusion nucleus and the endosperm nuclei (Figs. 26–28). A similar condition has been reported in *Tetragonia expansa* and *Mesembrianthemum linguiforme* (Huber, 1924), *Mesembrianthemum pseudotruncatellum* (Schmid, 1925), several species of *Mesembrianthemum* investigated by D'Hubert (1896) and *Aizoon canariensis* (Dahlgren, 1927).

The pollen tube is large and thick and enters in the usual way through the micropyle. Actual double fertilisation has not been observed but remains of pollen tubes were present in all the ovules containing either the fertilised egg (Fig. 27) or young embryos.

Endosperm.—As seen from Fig. 28, the primary endosperm nucleus divides first and the fertilised egg follows soon after. The endosperm is free nuclear (Fig. 30) as in all other plants of this family that have been previously investigated. The nuclei are limited to a thin peripheral layer of cytoplasm but it is specially dense in the narrow chalazal pouch which continues to push its way down into the tissue of the nucellus until it reaches almost to the chalazal end of the seed. The embryo sac now assumes the form of an extensive horse-shoe-shaped cavity into which the embryo continues to grow, just as in *Mollugo nudicaulis* (Bhargava, 1934). Wall formation starts at the micropylar end and extends towards the chalazal region.

Embryo.—Fig. 28 shows the dividing egg. The first wall is transverse or slightly oblique (Fig. 29). Figs. 30–33 show further stages in the development of the proembryo. The terminal cell divides by a longitudinal wall (Fig. 33); this is followed by further transverse and longitudinal divisions resulting in a globular embryo (Fig. 34). Fig. 35 shows a more advanced stage where the two cotyledons have just been marked out and Fig. 36 shows a still advanced stage with the stem tip also distinguishable. All the cells of the rather peculiar suspensor, except the four or five just adjacent to the embryo, become broadened and divide irregularly thus giving it the form of a massive structure of many cell rows (Fig. 31). Dahlgren (1916) also found in many species of *Mesembrianthemum* and *Tetragonia* a suspensor formed of several cell rows. In *Mesembrianthemum crystallinum* (Woodcock, 1930) transverse divisions occur in the suspensor, producing a row of cells some of which later on divide longitudinally. According to Huber (1924), on the contrary, the lowest suspensor cell is strongly developed in *Mesembrianthemum* and the present writer found a uniseriate suspensor in *Mollugo nudicaulis*.

In the mature seed the inner layer of the inner integument and the outer layer of the outer integument become greatly thickened and hardened

as in *Mollugo nudicaulis* (Bhargava, 1934) and in plants of the allied family Phytolaccaceæ worked out by Lewis (1905) and Mauritzon (1934). Further, the cells of the outer layer of the outer integument become very much elongated and flattened. The outer layer of the inner integument finally gets crushed and disappears (Fig. 15). Both the layers of the aril remain unthickened but the cells of the outer layer become more or less elongated in the radial direction (Fig. 15).

In *Mesembrianthemum crystallinum* (Woodcock, 1930), *M. linguiforme* (Huber, 1924) and *Phytolacca americana* (Woodcock, 1924) only the outer layer of the outer integument becomes hard.

Discussion.

Following Engler and Prantl, most systematists regard the Phytolaccaceæ as the parent family from which other families included in the order Centrospermales have originated by development along somewhat different lines. In many respects all the families show remarkable similarities but it seems fairly certain that the Aizoaceæ has departed least from Phytolaccaceæ.

Stem anatomy.—The structure of the stem in a number of genera of both Aizoaceæ and Phytolaccaceæ resembles in having the same type of anomaly which results in the appearance of successive rings of vascular bundles in the pericycle.*

Floral organs.—The Aizoaceæ resemble the Phytolaccaceæ in their morphologically apetalous flowers. In the showy *Mesembrianthemums* the members of the outer series of stamens become petaloid forming a showy corolla-like series while in fact the true corolla is absent. In both families the perianth is 4-5 merous and frequently tubular. The stamens and carpels vary widely in number. In both cases the ovary is typically syncarpous and contains numerous ovules, but in Phytolaccaceæ each carpel contains a solitary ovule while in Aizoaceæ each carpel has a row of ovules.

Ovule.—In both these families the nucellus becomes large and massive due to periclinal divisions of the cells of the epidermis. There are always two integuments except in *Trianthema monogyna* where a third integument is also present. The micropyle is always formed by the inner integument. In the mature seed usually the inner layer of the inner integument and the outer layer of the outer integument become greatly thickened and hardened.

Usually there is a single hypodermal archesporial cell which cuts off a wall cell, but occasionally more than one archesporial cell is also present. In the five species of Phytolaccaceæ, worked out by Mauritzon (1934), usually

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* For details refer to Pfeiffer (1926) and Solereder (1908).

only three megaspores are formed, of which the micropylar contains two nuclei and only rarely a T-shaped tetrad of four megaspores is formed. Similar cases of three megaspores have been seen in *Trianthema monogyna* (Bhargava, 1935), *Mesembrianthemum eklonis* and *M. corymbosum* (Guignard, 1882) and *Mollugo nudicaulis* (Bhargava, 1934). In both the families the endosperm is nuclear and an accumulation of the cytoplasm and free nuclei is very commonly seen at the chalazal end of the embryo sac.

Summary.

1. The microspore mother cells are surrounded by a bi-nucleate tapetum, two middle layers and an endothecium. Cytokinesis occurs by furrowing. The microspores are arranged tetrahedrally. The microspore nucleus divides forming a large vegetative cell and a small generative cell which divides within the anther lobe to form two male cells.

2. The ovules are amphitropous and have two integuments. The inner integument is two cells thick except at the end forming the micropyle and the outer is three cells thick. An aril or third integument arises early and finally surrounds the ovule.

3. The cells of the nucellar epidermis divide freely, but those lying just above the embryo sac merely stretch out radially.

4. Usually a single hypodermal archesporial cell is present in the nucellus and this divides to form the primary wall cell and the megaspore mother cell. Occasionally two megaspore mother cells are also present.

5. After the two reduction divisions are over, three megaspores are formed of which the micropylar contains two nuclei. The chalazal megaspore functions.

6. The mature embryo sac is of the usual eight-nucleate type with ephemeral antipodals. Starch grains were seen in older embryo sacs.

7. The endosperm is free nuclear and the wall formation starts at the micropylar end.

8. The first division of the egg is by a transverse or slightly oblique wall. The suspensor is long and massive and consists of several cell rows. The embryo is of the usual dicotyledonous type.

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THE LIFE-HISTORY OF *OTTELIA ALISMOIDES* PERS.

BY S. K. NARASIMHA MURTHY, M.SC.,

Department of Botany, Central College, Bangalore.

Introduction.

PERHAPS the earliest study of this genus is that of Palm,[10] who investigated the development of the embryo sac in *Ottelia lancifolia*. He observed that it conforms to the *Helobiales*-type and that the basal portion of the embryo sac forms a large pouch with three antipodals seen persisting for a long time. In *Enalus acoroides* Svedelius[15] observed fairly large antipodals and a very big suspensor cell. *Vallisneria spiralis* has been subjected to detailed investigation from various points of view. Elfving[5] and Burr[1] reported the formation of trinucleate pollen grains and the poor development of the endosperm tissue. Wylie[18] has made an exhaustive study on the mode of its pollination. More recently, Rangasamy[11] has carried out a detailed investigation of its life-history and reports that no endosperm tissue is formed. Equally interesting and exhaustive work has been done on *Elodea canadensis* by Wylie[18] and Santos.[12] The former has investigated its life-history and the latter has described its sex chromosomes.

Material and Methods.

The material was collected in the vicinity of Bangalore. It is a completely submerged aquatic plant with leaves rolled inwards presenting a funnel shape. The flowers are solitary, axillary and come up to the water level by the elongation of the pedicel. The material was fixed in Bouin's fluid. Sections were cut at 8 to 10 microns and stained in iron-alum-hæmatoxylin.

Investigation.

Microsporogenesis.— The young anther which at first consists of a mass of homogeneous cells, shows a four-lobed appearance in cross-section. In the young stages the sporogenous cells cannot be distinguished from the surrounding tissue. Gradually the hypodermal archesporial cells are differentiated. They cut off a parietal layer which undergoes the usual development and forms an endothecium, a middle layer and an inner tapetum (Fig. 1). The tapetal layer consists of large uninucleate cells which gradually coalesce and form a periplasmodium (Fig. 13). Though Schürhoff[14] records that

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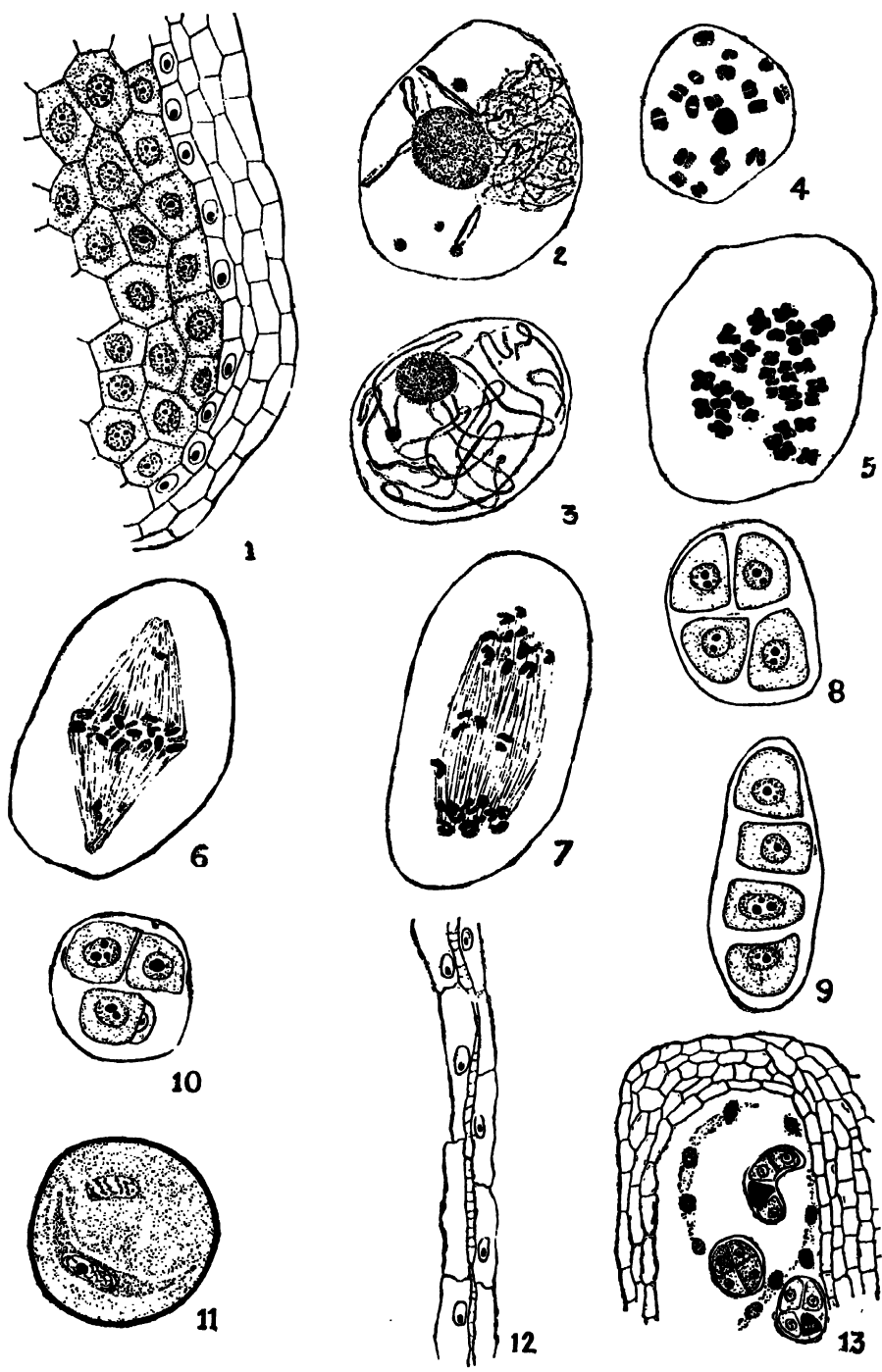
the periplasmodium formation is a general feature of this family, it has not been reported in *Vallisneria* by Rangasamy.[11] The middle layer persists for a long period till the time of dehiscence of the anther. The endothecium does not develop well (Fig. 12). A similar case is observed in *Vallisneria*, where the author further points out that special rigid outgrowths are developed from the exothecium.

The sporogenous cells divide a number of times and form the microspore mother cells. At first these are distinctly polygonal and undergo a prolonged period of rest (Fig. 1). During the resting period the chromatin is uneven but gradually it becomes uniform as synizesis sets in. The synizetic knot is loose with the nucleolus being always excluded from it (Fig. 2). The double nature of the thread can be easily made out in the loops thrown out from the synizetic knot. During the open spireme stage the double threads are still clear and they are even (Fig. 3). In diakinesis stage the gemini are short and do not exhibit the quadruple nature (Fig. 4), but at the heterotypic metaphase this feature becomes evident (Fig. 5). At this stage a few of the bivalents are precocious and will have migrated to the poles, while the rest are still at the equator (Fig. 6). The haploid number of chromosomes is 36 (Fig. 5). During the anaphase a few lagging chromosomes are observed (Fig. 7).

When the homotypic divisions are over, isobilateral tetrads of microspores are formed which may be arranged in a variety of ways. In addition to the normal type (Fig. 8) which is more common, the tetrads may be arranged either in a linear manner (Fig. 9) or a pair of them at right angles to another (Fig. 10). Similar cases of departure from the general arrangement have been recorded in a few cases. Wille[17] found that the tetrads were arranged indiscriminately in *Orchis mascula*, *Juncus* and *Typha*. Linear arrangement of microspores is common in *Asclepias* (Frye[6]).

Another noteworthy feature in *Ottelia alismoides* is the degeneration of some of the pollen grains even at the tetrad stage (Fig. 13). Many cases of degeneration of ripe pollen grains are also observed in the anther locule. Similar cases have been noticed in *Zostera* and a few *Cyperaceæ* (Lyon[8]).

The mature pollen grain has a tube nucleus and a generative cell (Fig. 11). In all the members of the *Hydrocharitaceæ* so far investigated only trinucleate pollen grains have been reported (Schürhoff[14]). The pollen grains have fairly well developed exine, though in some aquatic members as *Najas* and *Zannichellia* development of the exine is suppressed (Campbell[2]).

Ovule and the female gametophyte.- The ovary is trilocular with parietal placentation. The primordia of the anatropous ovules soon appear on the
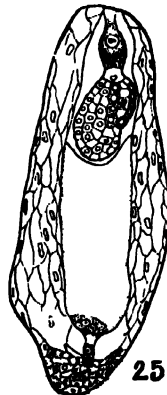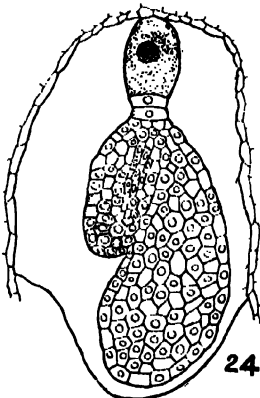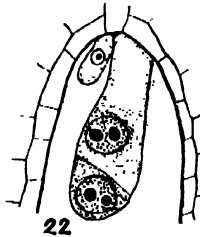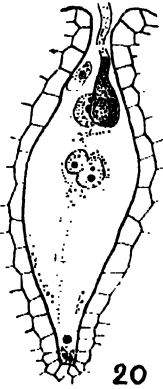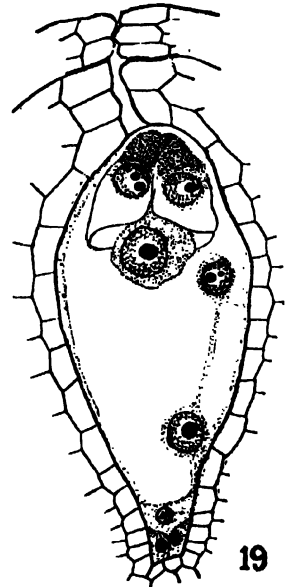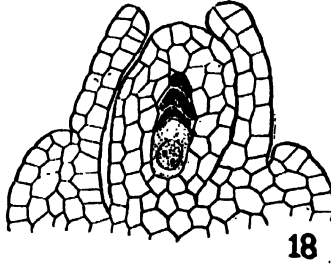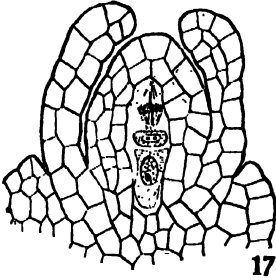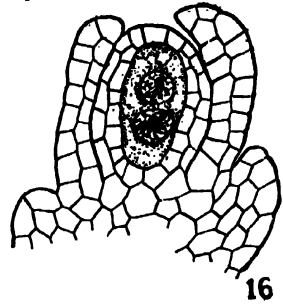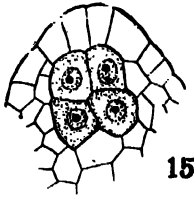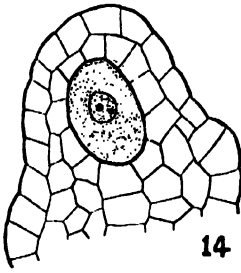
placental tissue. Each primordium consists of a mass of uniform cells at first. Gradually the archesporium which is hypodermal becomes situated deeper down by the division of the cells of the epidermal layer (Fig. 14). Sometimes a multicellular archesporium is also observed (Fig. 15). But even in the latter case only one develops ultimately. Schürhoff[14] reports that the occurrence of a single archesporial cell is a general feature of many plants of this family, while Coulter and Chamberlain[4] record that instances of monocots showing multiple archesporium are very few. But it has been noticed in *Lilium philadelphicum* by Chamberlain.[3] Guignard[7] has described its formation in *Ornithogalum pyrenaicum*.

In *Ottelia alismoides* the archesporial cell directly functions as the megaspore mother cell. In this respect it resembles *Vallisneria spiralis* (Rangasamy[11]). This observation is at variance with that of Schürhoff[11] who reports that the parietal cell formation is one of the general features of this family. Wylie[18] has also described its formation in *Elodea canadensis*.

The integuments appear early at the base of the nucellus as concentric rings of actively growing tissue. The large megaspore mother cell passes through the usual meiotic cycle and divides to form two nuclei with no cell wall between them (Fig. 16). The usual homotypic divisions follow, resulting in the formation of a linear tetrad (Fig. 17). In *Vallisneria spiralis*, Rangasamy[11] has observed the formation of only three megaspores, while in *Elodea canadensis* Wylie[18] records the formation of six megaspores sometimes.

Gradually, the functioning chalazal megaspore enlarges and the mitotic divisions follow. From the binucleate stage onwards, the embryo sac begins to grow in length with a large vacuole in the centre. The mature embryo sac is typically eight-nucleate (Fig. 19). The egg is more or less pear-shaped and has a large nucleus at the base surrounded by a dense mass of cytoplasm. The cytoplasm and the nucleus of each synergid is situated at its pointed micropylar end while the basal portion has a large vacuole. Both the synergids are bent in a concave manner at their vacuolate ends which form a chamber in which the egg is suspended (Fig. 19). Of the two polars, the antipodal one is slightly larger. The three nuclei at the lower end of the embryo sac are organised to form the antipodal cells (Fig. 19).

Fertilisation.—Polar fusion begins at the time of the entry of the pollen tube (Fig. 20). Syngamy takes place in a resting condition of both the nuclei. The polar nuclei are half fused by the time the second male nucleus enters into the combination (Fig. 21).

Embryogeny.—The fertilised egg divides forming a large basal cell and a terminal one. The former enlarges considerably and remains prominent

up to a late stage in the formation of the embryo. One of the synergids is also seen persisting for a long time (Fig. 22). After the formation of the three-celled proembryo the basipetal divisions commence thus conforming to the Sagittaria-type (Schaffner[13]). In Limnophyton obtusifolium also a member of the Helobiales, the author[9] finds a four-celled proembryo before the terminal cell divides. In Vallisneria spiralis (Rangasamy[11]) and Elodea canadensis (Wylie[18]) a four- to five-celled proembryo is formed before the basipetal divisions commence. The fully developed embryo has a large terminal massive cotyledon and a lateral stem tip situated in a depression (Fig. 24).

Endosperm.—The endosperm formation is rather interesting. The primary endosperm nucleus migrates towards the chalazal end of the embryo sac and divides, accompanied by a wall formation. Of the two chambers thus formed the micropylar chamber is very big in the early stages (Fig. 23). Later on, nuclear divisions follow in both the chambers resulting in the peripheral distribution of the endosperm tissue which is rather meagre. The micropylar chamber, though larger at first, is equalled in size by the rapid growth of the antipodal one. The latter becomes active, elongates and forms a pouch-like structure in which the antipodals are seen persisting for a long time (Fig. 25). The formation of a similar structure is reported in Elodea (Wylie[18]) where it is organised much earlier. This region, in the case of Ottelia alismoides, assumes a fairly aggressive haustorial rôle and feeds upon the chalazal tissue (Fig. 26).

Summary.

1. The microspore tetrads are arranged in isobilateral, linear or decussate manner. The tapetum forms a periplasmodium. The pollen grain has a conspicuous generative cell and a degenerating tube nucleus. The degeneration of some of the pollen grains is also observed. The endothecium is not well developed.

2. In the nucellus there is usually a single archesporial cell which directly functions as the megaspore mother cell. Occasionally, a multicellular archesporium is noticed.

3. The embryo sac is normal in which the antipodals are organised into cells.

4. The embryo is of the Sagittaria-type.

5. The primary endosperm nucleus divides accompanied by a wall formation. The upper chamber lodges the embryo and the lower one along with the persisting antipodals assumes a haustorial rôle. The endosperm tissue is feebly developed.

In conclusion the author considers it a pleasant duty to express his gratitude to Dr. M. A. Sampathkumaran for helpful suggestions and advice during the course of this investigation.

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S. K. Narasimha Murthy

EXPLANATION OF FIGURES.

Fig. 1. Longitudinal section of a young anther showing microspore mother cells and the different wall layers. × 900.

Fig. 2. Pollen mother cell in synizesis. × 1800.

Fig. 3. Pollen mother cell in open spireme. × 1800.

Fig. 4. Pollen mother cell in diakinesis. × 900.

Fig. 5. Pollen mother cell in heterotypic metaphase showing 36 bivalents. × 1800.

Fig. 6. Pollen mother cell in heterotypic metaphase showing two precocious chromosomes migrating to the poles. × 1800.

Fig. 7. Pollen mother cell in heterotypic anaphase showing a few lagging chromosomes. × 1800.

Fig. 8. Isobilateral tetrad of microspores. × 900.

Fig. 9. Linear tetrad of microspores. × 900.

Fig. 10. Decussate tetrad of microspores. × 900.

Fig. 11. Mature pollen grain showing the crescent-shaped generative cell and a tube nucleus. × 900.

Fig. 12. A portion of the anther wall at the time of dehiscence showing the ill-developed endothecium. × 900.

Fig. 13. A portion of the anther locule with three kinds of microspore tetrads; one degenerating microspore is seen in two of the tetrads; also tapetal periplasmodium. × 900.

Fig. 14. Longitudinal section of a young ovule showing a single archesporial cell. × 900.

Fig. 15. Longitudinal section of a young ovule with multiple archesporium. × 800.

Fig. 16. First division of the megaspore mother cell. × 400.

Fig. 17. Formation of the linear tetrad. × 400.

Fig. 18. The enlarging chalazal megaspore with three others degenerating. × 400.

Fig. 19. Mature embryo sac with the egg apparatus, the two polars and three antipodal cells. × 800.

Fig. 20. Entry of the pollen tube and polar fusion. × 560.

Fig. 21. Triple fusion. × 1800.

Fig. 22. First division of the fertilised egg with one persisting synergid. × 800.

Fig. 23. The embryo sac divided into two unequal chambers by the division of the primary endosperm nucleus; a three-celled proembryo is seen in the micropylar chamber. × 400.

Fig. 24. Late embryo showing the terminal cotyledon and the lateral stem tip. × 400.

Fig. 25. The enlarging chalazal and micropylar chambers. × 100.

Fig. 26. The chalazal haustorium and the persisting antipodals. × 400.

BLOOD PARASITES OF *CORACIAS B. BENGHALENSIS* WITH SPECIAL REMARKS ON ITS TWO TYPES OF *LEUCOCYTOZOON*.

By Col. I. Froilano de Mello

And

Emidio Afonso,

(Nova-Gôa).

AMONG the birds of the genus *Coracias* we find in Wenyon[1] : *Coracias indica* in whose blood Plimmer in 1912 and 1914 recorded a *Hæmoproteus*. *Coracias abyssinicus* in whose blood Carpano found a *Hæmoproteus* in 1913 in Eritrea, A. et M. Léger in 1914 a *Hæmoproteus* in 1925 (specimen from Abyssinia) ; A. et M. Léger a *Leucocytozoon* in 1914 in Senegal. *Coracias garrulus* in whose blood Danilewsky recorded *Trypanosoma avium* in 1885 in South Russia ; Cardamatis a *Hæmoproteus* in 1909 in Greece ; Wülker a *Hæmoproteus* in 1919 in Macedonia ; Danilewsky a *Hæmoproteus* in 1889 in South Russia ; Wülker a *Leucocytozoon* in Macedonia in 1919.

Our bird (one specimen shot at Corlim, department of Ilhas, identified by Dr. Baini Prasad) shows the following parasites :

(1) *Hæmoproteus.*—Sexual dimorphism shown by the tinctorial reactions of the protoplasm which stains blue in female gametocytes and is colourless or slightly yellowish blue on males. The young forms of female gametes are small and more or less ovoid. When grown up, beautiful halterides embrace the nucleus of the red cells, which is displaced at the periphery. The protoplasm of the female is alveolar and does not stain uniformly ; the blue tone is more pronounced on the poles and is lighter on the centre. Some specimens show the violet rings which we have found also in some other species of this genus. The halteride forms may be pointed with a kind of tail-like appendage, but when fully grown up they are broad, regular, lodging in their concavity the nucleus of the host cell. The pigment is yellow brown collected in minute granules or big dots and showing a tendency to collect on the poles. The nucleus of the female gametocytes is either compact or more or less granular. When free, the female gametocytes are roundish or oval, blue protoplasm, nucleus compact or irregular, pigment irregularly scattered over the body in clusters or isolated granules.

The male gametocytes show the same forms as the female ones, a little more irregular in young stages. The pigment shows the same appearance, perhaps more definitely collected on poles than in females in fully grown-up specimens. Nucleus under the form of an irregular spireme. When free, the male gametocytes are roundish, the protoplasm having a slightly violet bluish tone.

The red cell is slightly hypertrophied.

So many *Hæmoproteus* have been recorded in other *Coracias* that it is very difficult to autonomise our species without consulting the original papers of the authors who studied them. The way in which the female gametocyte stains, with the poles deep blue and the centre in lighter tone constitutes for us one characteristic of this parasite as well as the colour and the form of distribution of the pigment.

We note however that none of the authors who studied the *Hæmoproteus* of *Coracias* have named them : so rejecting the idea that it should be identified with *H. danilewsky*, as many authors have done concerning the Hæmoproteids of birds in general, we are in a position to classify the species as *Hæmoproteus coraciæ*, which will take a trinominal designation, if not entirely similar to those already described. So we present this sp. as *Hæmoproteus coraciæ benghalensis* sp. n. (var. nov ?).

20 microns

(2) *Leucocytozoa.* —Two species of *Leucocytozoon* have been found by us in this bird, distinct enough to be considered as separate species. Before describing them and making some remarks on a number of points which the study of these parasites suggests, we will refer to the literature already quoted in the description of our *Leucocytozoon chloropsidis*,[7] and point out some other facts from other papers at our disposal.

Coles[2] states that he has found *Leucocytozoa* in many birds, as jay, thrush, blackbird, pigeon, starling, moorhen and many others apparently all of the round type. He has never seen the spindle-shaped L. " It may be argued that the forms I have met with have been the spindle-shaped specimens which have become rounded off after the blood was taken from the body. I am convinced that this has not been the case (except perhaps in a few cases in which the bird had been dead some time) as films were made and fixed at once by osmic acid vapour, or a fresh drop of blood was covered with a cover-glass

and examined immediately and in no case has there been any approach to the appearance of a spindle-shaped body. "

As far as concerns this point of a transformation of the so-called spindle-shaped L. into round L., we have in this bird definite evidence that these two forms belong to two different species for the following reasons :

(a) the structure of the spindle-shaped L. is finely alveolar and it takes a more or less light blue tone to Leishmann or a light grey to Heidenhain's iron hæmatoxylin ; that of the round L. is very compact, uniformly deep blue (Leishmann stain), or very dark (hæmatoxylin) with some white circular spots giving the impression of vacuoles devoid of any substance ;

(b) the nucleus of the spindle-shaped L., central or sub-central, is long, oval or reniform, whilst that of round L. is circular, with a stronger nuclear membrane, and much smaller comparatively to the former.

(c) the forms of the spindle-shaped L. which become free never take the round, almost circular form of the second L. They are oval and show a structure entirely similar to the L. contained in those *cellules à cornes* of French authors.

" It is difficult to dogmatise as to the nature of the host cell ; generally it seems to be a leucocyte or an immature red cell" continues Coles. We prefer for the present not to advance any hypothesis on the nature of the cells. But we possess enough elements to state that contrary to the opinion of the authors who say that each of these L. attacks one special cell (see the literature quoted on the description of *L. chloropsidis*), both these species of L. attack the same kind of cells. Their nucleus has the same structure in both cases and moreover, between the forms contained in the *cellules à cornes* and the free forms of the same parasite we find all the transitional stages where sometimes the poles of the cells are missing (one or both), or so slightly stained, almost vanished, that it is difficult to detect their existence. Among these transitional forms we find somewhere the nucleus alone remains attached to the L. just as in the case of the second L. On these grounds we are convinced that both these species of L. attack the same cell and that the second one has the property of lysing the cell protoplasm much more rapidly and completely than the spindle-shaped L., which however does it too, to a certain extent.

From the description and microphotographs illustrating the paper it seems that Coles has been happy enough to observe the divisional forms of the L. of the thrush.

In 1914 Laveran and Mesnil described as *Hæmamæba liothricis* (in Wenyon's book *Leucocytozoon liothricis*) a L. of the found type which for the convenience of description we will name *Type B*. There is nothing peculiar in this L. but the remarks of the authors concerning the nomenclature should be recorded : " Danilewsky a employé en 1889, le mot *Leucocytozoa* pour désigner d'une façon générale les parasites ayant leur siège dans les leucocytes, sans créer sous ce titre un genre bien défini. Depuis lors, on a appliqué la dénomination de Leucocytozoon à des Protozoaires qui appartiennent à des genres différents : Hémamibes des oiseaux et Hémogregarines des Mammifères parasitant des leucocytes et on a attribué à des genres différents des hématozoaires de même genre, suivant qu'ils parasitaient des hématies ou des leucocytes (Hémogrégarines des mammifères) ; il est demontré que certains hématozoaires des oiseaux classée comme L. parasitent des hématies et que chez certains animaux à sang froid, la même hémogrégarine parasite tantôt les hématies et tantôt les leucocytes. La confusion est donc complète. "

The authors insist that this parasite lives in the red cells and add : "la conclusion à tirer de ces faits est que les hématozoires endocellulaires ne doivent pas etre classés suivant les cellules qu'ils parasitent, mais d'apres leurs caracteres morphologiques et évolutifs et que le genre Leucocytozoon *dont la création a été attribuée à tort à Danilewsky* (italic ours), ce nous semble, n'a pas sa raison d'être."

Commes[4] describes a spindle-shaped L. in *Astur badius var sphenurus*, which he names *L. Martyi*.

Moldovan[5] describes in the smears of organs "à coté des gamètes typiques, des formes incluses dans des cellules mononucléaires (lymphocytes ou erythroblastes) (?) qui d'apres leurs caractères devaient être interprétées comme des schizontes jeunes du *Leucocytozoon Ziemanni.* ... Les formes les plus jeunes entrent déjà en rélation avec le noyau de la cellule parasitée, soit en s'accolant à lui, *soit en pénétrant partiellement à son intérieur ou en l'entourant sans le déformer* (italic ours). À un stade de developpement plus avancé d'un certain nombre de *ces trois variétés de schizontes* (italic ours), en treuve le noyau du parasite en division. Les divisions nucléaires se succèdent rapidement et on arrive à trouver des schizontes possédant, comme je viens de dire, plus de 30 noyaux. Nous n'avons pas encore constaté la formation de mérozoites."

Wenyon[1] remarks that this process is similar to that described by Coles and by Knuth and Magdeburg in *L. anseris* in Germany as occurring in the internal organs, either within mononuclear cells or free in the plasma and

that the forms observed by these authors have been probably correctly interpreted as schizonts.

Marcel Léger[6] describes : *L. gentili* from *Fringilla petronia* : "ce L. se trouve inclus dans des celleles hôtes arrondies. C'est un parasite des leuco-cytes mononucléaires." And this classification of the host cells as well as the description of their form as *arrondies* is made, despite that " de la cellule hôte on ne perçoit, dans la majorité des cas, que le noyau rejeté à la peripherie, déformé, bosselé, allongé et bordant le parasite sur un tiers ou même une moitié de sa périphérie." With such elements we do not know how it was possible to describe the host cells as *roundish* and classify them as *mono-nuclears* as the author does again when referring to the male gametocyte. He says : "le noyau est le seul vestige de la cellule hôte, est bien plus dé-chiquété et se colore moins bien que celui d'un *mononucléaire* (italic ours) hébergeant une forme femelle."

Describing le *L. marchouxi* from *Turtur auritus* there are at least some elements to try to classify the host cell. "la cellule hôte, arrondie, reste presque toujours intacte. Le protoplasma coloré en *gris bleu* (italic ours) par le Giemsa apparait en bordure du parasite. Le noyau aplati et refoulé, se colore normalement ; il est certainement atrophié mais non en Karyolyse."

Or *grisbleu* is also the colour which takes the spindle-shaped poles of the host cell harbouring the L. of the A type. Will not then the host cells in both cases be of the same nature, differently deformed by the specific action of these types and will it not be more advisable to classify these types of L. as different genera ?

We will now describe our *Leucocytozoa*, found either on the blood, or in the smears of the lungs, more abundant in these smears.

Type A.—Ovoid, included in a fusiform cell, whose poles are greyish blue to Romanowsky and slightly stained at Heidenhain's iron hæmatoxylin. The nucleus of the host cell is attached to one side of the parasite which on this account has sometimes a bean-shaped form. But its real form is ovoid as we can see either in free forms or in those where the nucleus of the host cell occupies a central position *vis-à-vis* of the parasite. Many forms have been seen where the host cell has lost one or both poles, reduced only to the nucleus.

Protoplasm constituted by a fine alveolar net well marked with iron hæmatoxylin. At Romanowsky it stains blue, but lighter than in Type B. Sometimes large vacuoles are also seen, but the structure of the protoplasm, framed on this general type shows so many individual variations that it is impossible through it to try to find out sexual dimorphism.

We have found in one specimen only, among hundreds studied, a lot of very small rose granulations around the nucleus.

Sexual dimorphism is distinguished by the structure of the nucleus, recognisable not to Romanowsky but to iron hæmatoxylin. Its form is ovoid or bean-shaped, its position central or sub-central. There are two types of nuclei : one small with karyosome and centriole or only with centriole— it belongs to the female gametocyte ; the other, much larger, often oval, elongated, or even bean-shaped, filled with granules not strongly siderophyl, and without any trace of centriole—it belongs to the male gametocyte.

The proportion of this *Leucocytozoon* to the other is from 35 to 5. We have met only adult gametocytes. No young form, no schizogonic stage was seen. Ratio of breadth to length 2/5 in intracellular forms and 4/7 in free forms.

20 microns
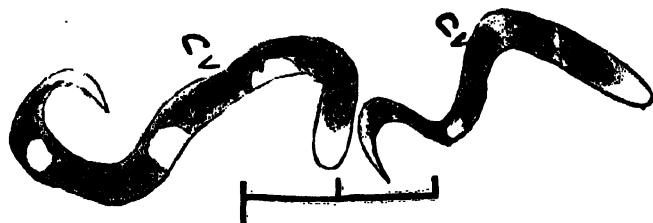
Coracias b. benghalensis

Type B.—Spherical, attached to the nuclear substance of the host cell from which no other structure is seen. The constitution of this host cell nucleus seems entirely similar to that which lodges the Type A.

Protoplasm compact, deep blue to Romanowsky, much darker than that of Type A to iron hæmatoxylin. No alveolar net has been evidently seen, but many vacuoles, generally small having sometimes larger dimensions almost white and not stainable at all appear in the protoplasm. As all specimens seen had the same tone of coloration and the nucleus with karyosome, it seems to us that we have found only one type of gametocytes. Forms of different sizes, the free ones absolutely spherical and not ovoid as in Type A.

The study of these Leucocytozoa shows that we are definitely dealing with two species autonomous and independent and it convinces us that while the first type may be classified as *Leucocytozoon* (genotype universally accepted: *L. ziemanni*), the second one has, as all the parasites of this type, enough characters to constitute an independent genus to which the name of Marcel Léger should be attached. For the present we do not advance more on this line, hoping for further material to settle this point.

I. *Froilano de Mello*
and
Emidio Afonso
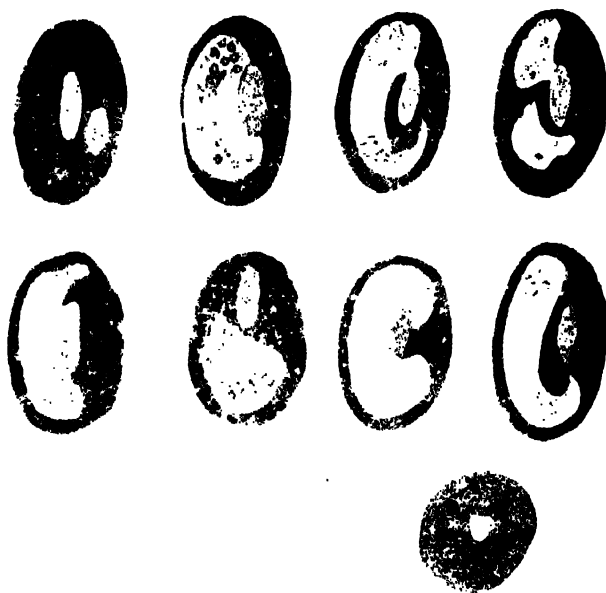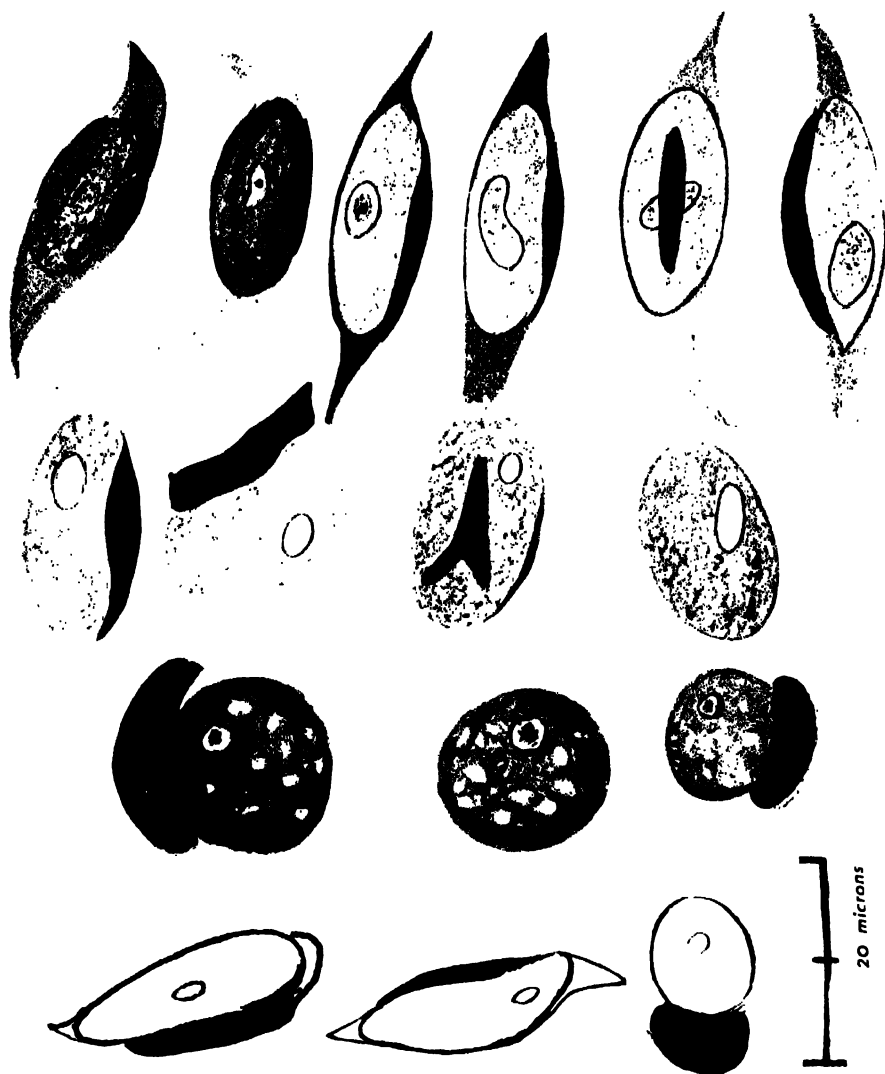
Coracias b. benghalensis L.

For classifying these parasites we are not aware of the characters of the I, of *Coracias abyssinicus* and of *Coracias garrulus*. As no specific name is registered in Wenyon, always so careful to record all specific names, it seems that we are allowed to give the following nomenclature :—

Type A—*Leucocytozoon coraciæ benghalensis.*

Type B—*Leucocytozoon* (or new genus to be created ?) sp.

(3) A *microfilarium* was present in such a scanty number, that we have found only four specimens in all the slides examined. Provided with a sheet. Anterior pole rounded. Posterior pole tail like, free on its last part from the nuclear mass which begins at six to seven microns from the anterior pole, as a compact mass. Three spots, the median (situated sometimes a little behind) in V or crescent form. In the middle of the body there is a very regular ovoid body, stained in rose by Leishmann and corresponding to the central Viscus of Manson. The caudal spot oval, very regular. Length 50-55. Breadth 4—5 microns.

N. B.—One specimen of *Coracias benghalensis* shot at Diu (identified by Mr. S. H. Prater from Bombay) did not show any parasite at all.

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STUDIES IN *DOLICHOS LABLAB* (ROXB.) AND (L.)— THE INDIAN FIELD AND GARDEN BEAN. II.

By G. N. Rangaswami Ayyangar, B.A., I.A.S.,
Agricultural Research Institute, Coimbatore,

AND

K. Kunhi Krishnan Nambiar, B.Sc.Ag.,
Millets Breeding Station, Coimbatore.

Seed Coat Colours—A Case of Linkage.

In *Dolichos lablab* (Roxb.) and (L.) there are three major seed coat colours, Khaki, Chocolate and Black, occurring in both field and garden varieties. In addition to these three colours there is a fourth colour, Buff, which has so far been met with in field varieties only. In this Buff, the colour of the seed coat is Buff except at the micropylar zone which is colourless and white.

In each of the three seed colours Khaki, Chocolate and Black, the colour may be present either over the whole of the seed coat or be restricted to portions thereof. The commonest instance of restriction in the manifestation of these colours is in most of the white grains in which the colour is confined to the micropyle, edging the raphe and concentrating again at the caruncle. From the caruncle there is a spur of colour ending in a fork. Near the caruncle there is a small patch of dotted colour on the body of the seed coat. This restriction of colour to the above zone may occur in all the three colours. There are thus three kinds of white grains with the above restricted colour pattern, which for the sake of convenience are designated Micropylar Khaki, Micropylar Chocolate and Micropylar Black. No varieties coloured Khaki, Chocolate or Black have been met with in ·hich the micropyle is white. It may be noted that there is a variety of *lablab*, Burman in origin, which is white and in which there is no micropylar colour, and this type of white is not considered in this article.

In the case of the Buff seed, however, a restriction of colour as explained above has not been met with. We do not therefore have a Micropylar Buff with a white seed coat, similar to those existing in the colours, Khaki, Chocolate and Black. A Buff seed coat colour and an absence of colour at the micropylar zone are the characteristics of this Buff coloured seed.

Each of the whole colours Khaki, Chocolate and Black has proved a simple dominant to Buff. Crosses between Buff and each of these three colours have given first generation plants in which the Buff characteristics were suppressed. In the second generation simple monohybrid ratios were

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The Coloured Plate will appear in the
next issue.

obtained between the whole colours Khaki, etc., and Buff with its colourless micropyle (Tables I, II and III).

TABLE I. *Buff* × *Khaki* (F_1-*Khaki*).

F_2 Family No.	Khaki	Buff
D. L. 1026 ..	78	21
„ 1027 ..	91	29
„ 1028 ..	70	26
Total ..	239	76
Expected, 3 : 1 ratio ..	236·25	78·75

TABLE II. *Buff* × *Chocolate* (F_1-*Chocolate*).

F_2 Family No.	Chocolate	Buff
D. L. 784 ..	100	32
„ 808 ..	64	18
Total ..	164	50
Expected, 3 : 1 ratio ..	160·5	53·5

TABLE III. *Buff* × *Black* (F_1-*Black*).

F_2 Family No.	Black	Buff
D. L. 789 ..	68	19
„ 791 ..	54	20
„ 799 ..	57	16
„ 802 ..	52	17
Total ..	231	72
Expected, 3 : 1 ratio ..	227·25	75·75

A factor designated K exists in all the three colours Khaki, Chocolate and Black and this factor is absent in Buff. This factor K brings about the colour Khaki (the basic colour for Chocolate and Black). Along with the production of Khaki colour, this K colours the micropylar zone.

A second factor Bf is responsible for the presence of colour in the body of the seed coat, barring the micropylar zone. When this is present the colour of the seed coat is Buff. With K this gives the wholeness (Body and Micropyle) to the colours Khaki or Chocolate or Black. A monogenic difference has been found in segregations between whole colour and micropylar colour in the three colours Khaki, Chocolate and Black. The three following tables summarise the experiences met with (Tables IV, V and VI.)

TABLE IV.

Micropylar Khaki × Khaki (F_1—Khaki).

F_2 Family No.	Khaki	Micropylar Khaki
From D. L. 602 ..	49	15
„ „ 604 ..	49	14
D. L. 862 ..	30	11
„ 865 ..	27	7
Total ..	155	47
Expected, 3 : 1 ratio ..	151·5	50·5

TABLE V.

Micropylar Chocolate × Chocolate (F_1—Chocolate).

F_2 Family No.	Chocolate	Micropylar Chocolate
From D. L. 603 ..	11	2
„ „ 829 ..	24	14
„ „ 1024 ..	25	6
Total ..	60	22
Expected, 3 : 1 ratio ..	61·5	20·5

TABLE VI.   *Micropylar Black × Black (F₁ - Black).*

F₂ Family No.	Black	Micropylar Black
From D. L. 824 ..	22	8
„ „ 841 ..	14	4
„ „ 843 ..	26	8
„ „ 850 ..	58	18
„ „ 852 ..	28	8
Total ..	148	46
Expected, 3 : 1 ratio ..	145·5	48·5

The two sets of monogenic segregations recorded above of $K : k$ and Bf : bf are simple by themselves. An interesting combination of this experience arises when the Buff with its colourless micropyle is mated with any of the three other colours restricted to the micropyle. The first generation gives a whole colour, Khaki, Chocolate or Black according to the micropyle concerned, but in the second generation the segregation is a 2 : 1 : 1 of $K Bf : K bf : k Bf$.

Khaki	:	Micropylar Khaki	:	Buff
or Chocolate	:	Micropylar Chocolate	:	Buff
or Black	:	Micropylar Black	:	Buff

The genotype k bf with an all-white seed coat was absent proving that K bf and k Bf are absolutely linked (*vide* Tables VII, VIII and IX).

TABLE VII.   *Buff × Micropylar Khaki (F₁- Khaki).*

F₂ Family No.	Khaki	Micropylar Khaki	Buff
D. L. 113 ..	79	39	32
„ 114 ..	125	55	57
„ 497 ..	29	16	13
„ 498 ..	13	4	6
„ 499 ..	18	6	8
„ 508 ..	69	30	27
„ 509 ..	74	44	51
„ 510 ..	49	34	22
„ 511 ..	53	24	33
„ 545 ..	95	54	43
„ 546 ..	89	43	38
„ 547 ..	80	32	32
„ 548 ..	77	46	37
Total	850	427	399
Expected, 2 : 1 : 1 ratio	838	419	419

TABLE VIII.   *Buff × Micropylar Chocolate (F_1—Chocolate).*

F_2 Family No.			Chocolate	Micropylar Chocolate	Buff
D. L. 553	..	..	123	57	54
,, 551	..	..	94	53	45
,, 555	..	..	100	46	49
,, 556	..	..	98	53	42
,, 558	..	..	110	67	51
,, 559	..	..	110	60	54
,, 560	..	..	89	39	47
Total	..		724	375	342
Expected, 2 : 1 : 1 ratio	..		720·5	360·25	360·25

TABLE IX.   *Buff × Micropylar Black (F_1—Black).*

F_2 Family No.			Black	Micropylar Black	Buff
D. L. 483	..	..	43	18	17
,, 710	..	..	40	19	28
,, 711	..	..	20	16	11
,, 717	..	..	12	7	7
,, 718	..	..	16	9	6
Total	..		131	69	72
Expected, 2 : 1 : 1 ratio	..		136	68	68

A factor Ch with K gives the Chocolate colour and when there is a dihybrid segregation between the seed colours Chocolate, Khaki and Buff the simultaneous segregation for whole colour to micropylar colour is consistent with the genetic hypothesis that K bf and k Bf are absolutely linked and the phenotypes Chocolate, Khaki, Micropylar Chocolate, Micropylar Khaki and Buff are realised in the expected proportions 6 : 2 : 3 : 1 : 4 (Table X).

TABLE X.

Buff (*ch*) × *Micropylar Chocolate* (F_1 *Chocolate*).

F_2 Family No.	Chocolate	Khaki	Micropylar Chocolate	Micropylar Khaki	Buff
D. L. 526 ..	74	21	34	11	46
„ 528 ..	61	14	20	9	40
„ 529 ..	101	36	34	17	51
„ 530	95	30	45	22	65
„ 531 ..	62	22	34	9	32
„ 532 ..	53	21	27	14	44
„ 533 ..	38	13	15	12	28
„ 534 ..	131	69	74	24	98
Total ..	618	226	283	118	407
Expected, 6 : 2 : 3 : 1 : 4 ratio	619.5	206.5	309.75	103.25	413

Summary.

There are four seed coat colours in *Dolichos lablab*, namely, Black, Chocolate, Khaki and Buff. Khaki is the basic colour for the other two colours Chocolate and Black. In Buff, the body of the seed coat is coloured Buff, but the micropylar zone remains white. In the first three colours, the colour may be either on the whole of the seed coat or confined to the micropylar and caruncular zone.

A factor K has the effect of producing the Khaki colour and colouring the micropylar zone. A factor Bf has the effect of producing the Buff colour and colouring the whole of the seed coat except the micropylar zone. It is found that K bf and k Bf are absolutely linked.

AN ASYNAPTIC MUTANT IN RICE
(*ORYZA SATIVA*).

By S. Ramanujam, B.A. (Hons.),

AND

N. Parthasarathy, B.A., B.Sc.,

Agricultural Research Institute, Coimbatore.

1. Occurrence and Description.

In the seed multiplication block of one of the Coimbatore strains, Co. 4, a sterile plant was noticed in the season of 1932–33. It looked slightly more erect and shorter than the others with non-emergent panicles and continued to grow and produce fresh tillers when the rest had stopped growth and ripened their ears. The flower opening was not normal in this plant and the anthers were considerably reduced and non-dehiscent. The pollen was found to be all aborted (Plate IV). The ovaries for all external appearance looked normal but would not set seed even when pollinated with normal *diploid* pollen. The plant was propagated vegetatively over two or three seasons and nearly a thousand ovaries had been cross-pollinated without any success. It would appear that ovule sterility is nearly as complete as pollen sterility. The plant resembled the parents in all its pigment and morphological characters and should evidently have arisen as a mutant.

This plant closely resembled another mutant designated 'male sterile' in external characters but differed from it in its cytological behaviour (Ramanujam, 1935). The sterility in the case of the present mutant has been found to be due to lack of pairing of homologous chromosomes at meiosis.

Similar asynaptic plants have been noted in Maize (Beadle, 1930), Nicotiana (Clausen, 1931) and Datura (Bergner, Cartledge and Blakeslee, 1934). In these cases the mutations appeared as heterozygotes which on breeding showed the mutant character to be a simple recessive to the normal. In Maize, it was further possible to confirm the inheritance by artificial crosses, as about 10% of the ovules set seed on being crossed with normal pollen. In rice, artificial crossing, to study the inheritance of this character has been unsuccessful, and heterozygous individuals occurring spontaneously have not been met with so far. The meiotic behaviour of this mutant described in this note, closely resembles that of the other asynaptic plants

and this would appear to confirm the presumption that this mutant is gene-tically similar to those reported in other genera.

2. *Cytology.*

(*a*) *Materials and Methods.* - Studies of meiosis in the asynaptic plant were largely confined to microsporogenesis as it was difficult to get suitable preparations for a study of megasporogenesis. Aceto-carmine smears of pollen mother cells and material sectioned in paraffin and stained with Iron-Alum-Hæmatoxylin and Iodine-Gentian-Violet were largely used for the study and drawings were made with the aid of a camera lucida at 2,000 and 600 diameters and reduced to ⅜ the size in reproduction.

(*b*) *First Meiotic Division.*—The youngest microsporocytes of the asynaptic plant exhibited 'synizesis' and resembled those of the normal. When however, the synizetic knot unloosened and the chromatin threads condensed to form the diakinetic chromosomes, no pairing was visible and later instead of the twelve bivalent chromosomes characteristic of normal plants, twenty-four univalents appeared at the stage correspond-ing to diakinesis (Fig. 1). At metaphase (Fig. 2) no closed bivalents were seen but chromosomes were sometimes noticed loosely attached at one end only. A true reduction division was absent and the twenty-four univalent chromosomes were irregularly distributed in the pollen mother cells at first anaphase (Figs. 3 to 7). At this stage the behaviour of the chromosomes, in several respects, resembled that of the rice *haploid* (Ramiah, Parthasarathy and Ramanujam 1933). The chromo-somes were distributed to the poles in all combinations like 1+23, 2+22 and so on to 12+12. In many cases the distribution of the chromosomes was either throughout the cell or on the spindles only. The spindles were of different sizes, shapes and number and these probably determine the distribution of the chromosomes at first anaphase. Such a connection between the spindles and chromosome distribution has already been sug-gested by Bergner and others in Datura. Some spindles were short and broad and these evidently determined such distributions as 0+24, 1+23 and 2+22. Others were long and they extended either lengthwise (Fig. 9) or in a crescent-shaped manner (Fig. 8) from pole to pole. In such cases, the chromosomes lagged at the plate and formed a third group more easily than when the spindles were short. Although one bipolar spindle was formed in most cases, tripolar and occasionally supernumerary and even split spindles (Fig. 10) were noted causing diverse distri-bution of the chromosomes at first division. Sometimes the chromosomes strayed into the cytoplasm and failing to be included in the daughter nuclei formed independent nuclei (Fig. 11). As a result of these irregular

distributions, instead of two equal cells associated with the end of first
division in normal plants, two cells, more often unequal than equal, and
even three cells were formed.   Occasionally a portion only of the cell was
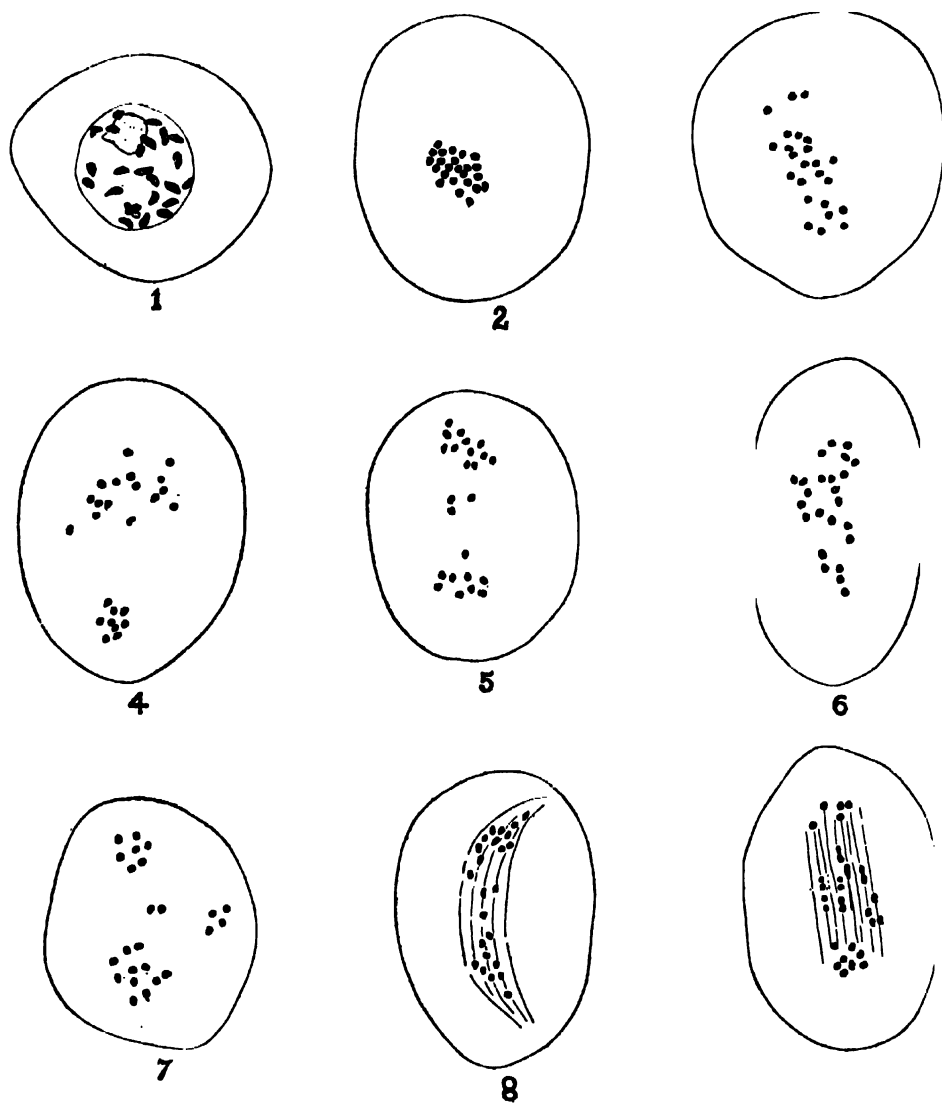
pinched off while all the chromosomes collected together in a single nucleus
in the pollen mother cell amounting to non-reduction.   In rare instances,
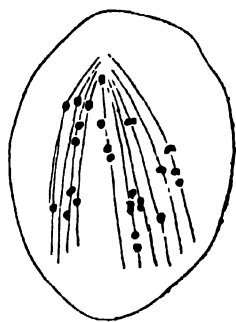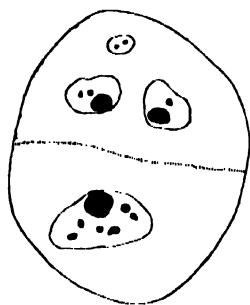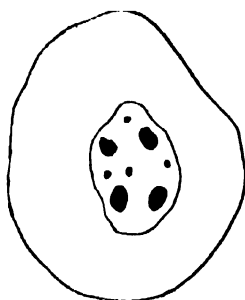
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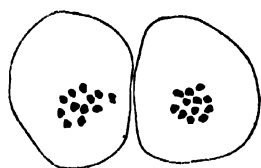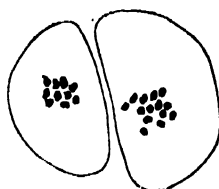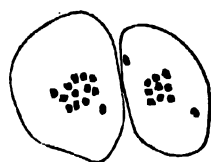
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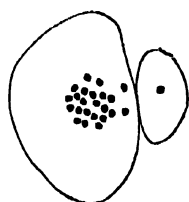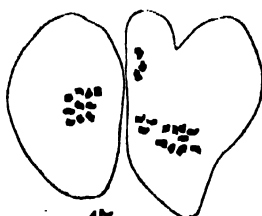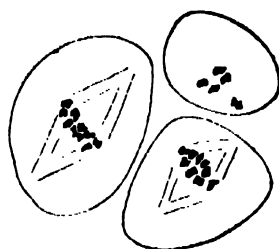
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failure of reduction and formation of a single nucleus at the end of the first division was also noticed (Fig. 12). Additional nuclei in daughter cells were common at this stage. Very occasionally the cells resulting from the first division included nuclei that appeared to be degenerating. This phenomenon has been noticed by Beadle in Maize. Splitting of univalents so characteristic of inter-specific and inter-generic hybrids was occasionally observed in some cells (Fig. 9). Such cases have also been noticed in the asynaptic plants of Maize and Datura. Similar phenomenon has also been observed in the rice *haploid* and *triploid* (Ramiah, Parthasarathy and Ramanujam, 1933). Giant cells, the result of coalescence of a number of pollen mother cells, with a large number of chromosomes assorting on several spindles, were noticed in aceto-carmine smears examined in 1932-33 season. But these, however, were not noted in 1933-34. It is possible that the season has some influence, as has been noticed with regard to the fertility of pollen in *tetraploids* (Ramiah, Parthasarathy and Ramanujam, 1935). Such plasmodial masses have been recorded by Beadle and McClintock (1929) in maize and Gaines and Aase (1926) in wheat.

(c) *Second Meiotic Division.* The second division was generally normal. But the cells were, however, commonly aberrant as a result of irregularities in the first division. Some of the cells in second metaphase are figured in Figs. 13 to 18 which show the irregular distribution of chromosomes at first division. Although cells with varying number of chromosomes were to be seen at this stage, those with 11+13, 12+12 and 14+10 were the most common. Supernumerary spindles and lagging chromosomes were sometimes also observed at the second division (Figs. 19 and 20). Occasionally, single cells with twenty-four unsynapsed chromosomes in the stage corresponding to diakinesis, were noted in anthers in which the majority of cells were undergoing second division. These probably are the result of ameiosis (suggested by Beadle for maize) which leads to the formation of unreduced spores. That unreduced spores also result from failure of first division and the splitting of univalents at second division was apparent in rare cases. Diminutive chromosomes recorded in maize were not noticed in rice.

(d) *Tetrad of Spores.*—Normally a microsporocyte is divided into a quartet of cells, the nucleus of each containing a set of twelve chromosomes. Here, as a result of the two irregular divisions, very few pollen mother cells showed a tetrad of equal sized spores characteristic of normals. Microspores and microcytes of varying size and number were formed (Figs. 21 to 26). The spores soon degenerated and a mature anther contained only shrivelled and apparently empty pollen grains.

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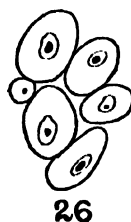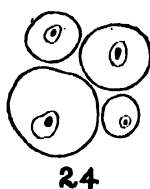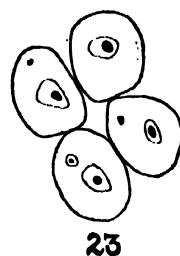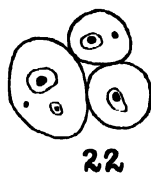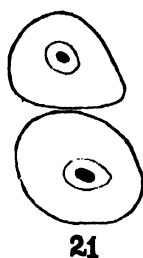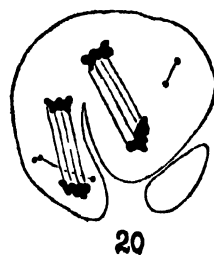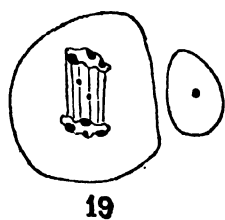
3. Discussion.

In general meiotic behaviour this mutant resembles those where a single recessive gene has been found to cause asynapsis at metaphase in Maize, Datura and Nicotiana. With regard to lack of bivalents at metaphase, the rice mutant resembles Datura but it differs from Nicotiana and Maize where varying number of bivalents has been noted to occur. While the asynaptic

plants of Datura, Nicotiana and Maize have given rise to some progeny either through their pollen or ovule, that of rice has not set any seed so far. Pollen of asynaptic plants of Datura used on the ovaries of $4n$ plants have given rise to *tetraploids* while normal pollen on maize asynaptic plants have yielded *triploids*. The pale steriles in Nicotiana have given rise to *trisomics* and *monosomics* when pollinated with normal pollen. Thus it is seen that such genetic factors as the one responsible for asynapsis afford an additional mechanism, besides many others already known, for the formation of *auto-polyploids* and *polysomics*. It is possible that such forms may arise from the asynaptic plant of rice which is being continually propagated vegetatively and crossed with normal plants reciprocally.

4. Summary.

An asynaptic mutant occurring in the bulk crop of one of the Coimbatore strains, Co. 4, is described and observations of its meiosis reported.

This plant closely resembles another mutant of rice designated 'male sterile' in external appearance, but differs from it in its cytological behaviour.

Meiosis in the microsporocytes of the asynaptic plant is characterised by complete failure of synapsis at metaphase and the presence of univalents leading to irregular distribution of chromosomes at first division. Splitting of univalents, presence of lagging chromosomes and supernumerary spindles are some of the other irregularities of this division.

Second division is comparatively more regular but is not free from irregularities like the presence of multiple spindles and lagging chromosomes.

Microspores of different numbers and sizes are formed in each pollen mother cell, as a result of the irregular divisions and these soon degenerate into shrivelled pollen grains.

Acknowledgments.

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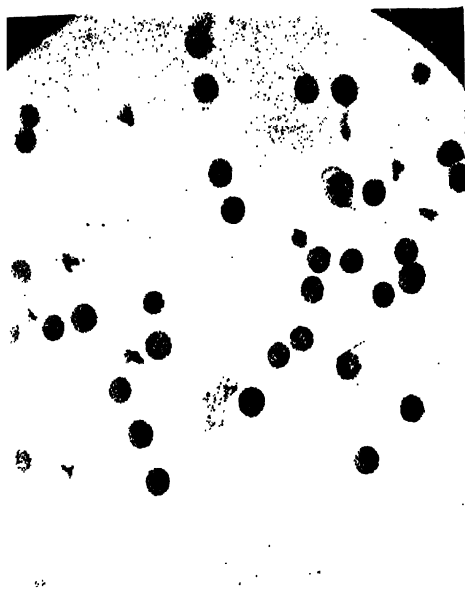
a   b

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EXPLANATION OF PLATE AND FIGURES.

PLATE I.—Pollen of normal (*a*) and asynaptic (*b*) plants (Aceto-carmine smears. ✕ 150).

FIG. 1. Stage corresponding to Diakinesis in the asynaptic plant (Gentian violet. ✕ 2000).

FIG. 2.—Metaphase (Gentian violet.).

FIGS. 3–7.—First anaphase showing irregular distribution of chromosomes (Gentian violet.).

FIG. 8.—Crescent-shaped spindle at first division (Gentian violet.).

FIG. 9.—A straight spindle with univalents splitting (Gentian violet.).

FIG. 10.—A split spindle (Gentian violet. ✕ 2000).

FIG. 11. Pollen mother cell after first division showing additional nuclei (Gentian violet. ✕ 2000).

FIG. 12.—Pollen mother cell where, after the first division, a single nucleus is formed (Gentian violet. ✕ 2000).

FIGS. 13–18.—Second metaphase showing irregular distribution of chromosomes in the two cells (Gentian violet. ✕ 2000).

FIG. 19.—Second anaphase with lagging chromosomes (Gentian violet. ✕ 2000).

FIG. 20.—Second anaphase showing supernumerary spindles (Gentian violet. ✕ 2000).

FIGS. 21–26. Different kinds of microspore groups (Aceto-carmine smears. ✕ 600).

ON THE MORPHOLOGY OF A NEW GENUS OF TREMATODE PARASITE OF THE KINGFISHER FROM LUCKNOW.

BY GOBIND SINGH THAPAR, M.SC., PH.D. (LOND.)

AND

MAKUND BEHARI LAL, M.SC. (LUCK.)

(*From the Department of Zoology, Lucknow University, Lucknow.*)

LÜHE (1909) created a subfamily, Psilostominæ, for the reception of the two genera— *Psilostomum* and *Psilochasmus*. Odhner (1913) described several new genera and added considerably to our knowledge of the group. He further considered the advisability of raising the group to the status of a family, Psilostomidæ. Travassos (1921) gave an account of a species of a new genus *Lyperorchis* under the family Psilostomidæ. Linton (1928) in his account of the Trematodes from North American birds recorded several new species of the genus *Psilostomum*, some of which do not appear to us to conform to the generic diagnosis. Bhalerao (1931) added yet another genus, *Testifrondosa*, from the intestine of pigs from India to the family under review. In the course of our investigations on the Trematode parasites of birds, we have recovered a few specimens from the intestine of a kingfisher, shot at Lucknow and these appear to be new to science. They are described as a new genus of the family. While reserving our criticisms to a later stage, we must say here that the discovery of this new genus under the family Psilostomidæ has revealed certain points of systematic value that necessitate the revision of our knowledge of this interesting group of Trematodes.

Psilorchis indicus N.G., N. Sp.

The body of this Trematode is long flattened, leaf-like and gradually tapers towards either end. It is 8·57 mm. in length and has a maximum breadth of 1·17 mm., which is at about the middle of the body near the ovary. The body is covered over with smooth cuticle and is devoid of any spines or scales.

The mouth is subterminally situated at the anterior end and is surrounded by a small oral sucker, which is not powerful. It is ·17 mm. by ·1 mm. in size. The ventral sucker is strong and powerful and is much larger than the oral sucker. It measures ·75 mm. by ·65 mm. and is situated at 2/9th

portion of the body from the anterior end. The genital pore opens in front of the ventral sucker, between it and the intestinal bifurcation.

The mouth leads into a short prepharynx, measuring ·18 mm. in length. The pharynx is globular, thick-walled and muscular and is ·18 mm. by ·16 mm. in size. This is followed by a small œsophagus, ·09 mm. long, and this latter bifurcates into two intestinal diverticula, running laterally to the posterior end of the body, ending blindly a little in front of it.

The excretory pore is situated at the posterior end of the body and leads into a short Y-shaped excretory bladder. The two horns of the Y lead into long excretory ducts which ramify in the body of the animal.

The ovary is oval and is situated slightly behind the middle of the body. It measures ·41 mm. by ·25 mm. From its posterior end arises a short narrow oviduct, which after a short course is met by the common yolk duct. Here it forms the öotype, which receives the Laurer's canal and the uterus also arises from it. There is no receptaculum seminis in these forms and the öotype is surrounded by a large number of unicellular shell glands.

1 mm.

FIG. 1.

·5 mm.
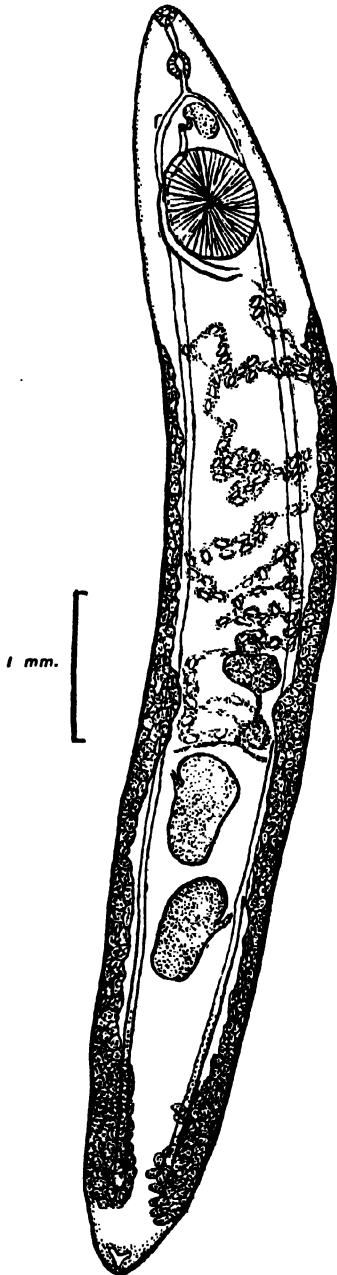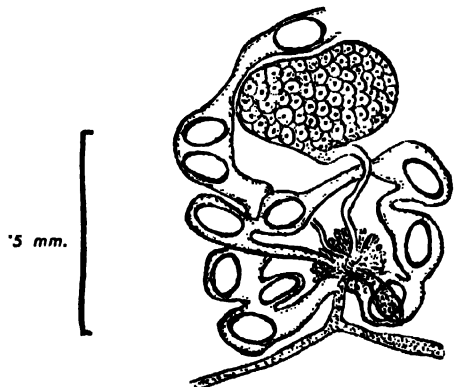
FIG. 2.

The vitelline glands are distributed behind the ventral sucker, as large number of diffused follicles laterally on either side of the body to the posterior end. These glands are chiefly extra-cæcal in position, but at places they project inwards interior to and covering the intestinal diverticula. At the posterior end of the body they predominantly become intra-cæcal but unlike other genera of Psilostomidæ and the Echinostomidæ they do not meet in the middle line. They all lead by their minute ducts into the lateral vitelline ducts on either side, and at the level of the öotype these lateral vitelline ducts join the transverse vitelline duct of its side and the two transverse ducts unite behind the öotype to form a median vitelline duct. This duct before entering the öotype collects its yolk into a small pear-shaped yolk reservoir.

The uterus arises from the right side of the öotype and forms an anterior loop round the latter. It then turns round behind it and in front of the anterior testis it turns forwards along the right side of the ovary. Then it follows a zig-zag course forwards to open at the genital pore in front of the ventral sucker as an elongated metraterm. The eggs are large oval structures measuring from ·125—·130 mm. by ·08—·1 mm.

There are two testes, more or less bean-shaped, situated behind the ovary and are tandem in position. Each testis is provided with an external lateral funiculus that leads forwards into a narrow vas deferens. The anterior testis

·5 mm.
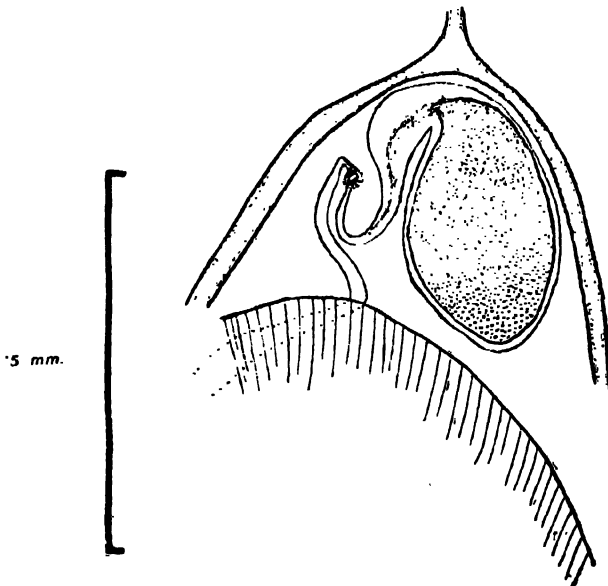
FIG. 3.

is situated behind the ovary at a distance of ·5 mm. and measures ·75 mm. long by ·42 mm. broad. The posterior testis measures ·75 mm. by ·36 mm. in size.

The vesicula seminalis, formed by the union of two vasa deferentia in front of the ventral sucker, is retort-shaped and anteriorly leads into a short ductus ejaculatorius and the cirrus. It opens at the genital pore close to the opening of the metraterm, the opening being slightly dextral, situated between the intestinal bifurcation and the acetabulum.

The characters of the genus *Psilorchis* may, therefore, be summed up thus :—

1. Leaf-like body, devoid of spines.
2. Ventral sucker much larger than the oral sucker.
3. Short Y-shaped excretory bladder.
4. Dextral position of the genital pore in front of the ventral sucker.
5. Ovary in front of the two testes, in the posterior half of the body.
6. Receptaculum seminis absent.
7. Uterine coils in front of the testes.
8. Vitellaria behind the ventral sucker and do not meet those of the other side posteriorly behind the testes.
9. Short cirrus and retort-shaped vesicula seminalis completely in front of the ventral sucker.

The genus described above belongs to the family Psilostomidæ, which also includes the genera *Psilostomum*, *Psilochasmus*, *Psilotrema*, *Apopharynx*, *Sphæridiotrema*, *Lyperorchis* and *Testifrondosa*. The present form differs from *Psilostomum* in the dextral position of the genital pore, in the position of the vesicula seminalis, in the short cirrus and in the absence of the posterior coalescence of the vitellaria. From *Psilochasmus* it can be distinguished in the shape of the posterior end of the body, the shape of the excretory bladder and the position of the vesicula seminalis. It can be differentiated from *Psilotrema* in the absence of the body spines, in the position of the ventral sucker, in the shape of the excretory bladder, in the length of the uterus with numerous eggs, in the position of the ovary and in the position of the genital pore. It can readily be distinguished from *Sphæridiotrema* and *Apopharynx* by the shape and position of the ventral sucker, the position of the ovary and the uterus and the genital pore. It differs from *Testifrondosa* in the absence of the receptaculum seminis, shape of the testes, the position of the vesicula seminalis, the shape of the ventral sucker and the presence of the prepharynx. The genus *Testifrondosa*, it may be mentioned, is unique amongst the members of the family Psilostomidæ in having a receptaculum seminis and it appears to

us that it should be removed from this family on this account.   We will urge for its inclusion under the family Allocreadiidæ with which it shows several other features in common.

The only other genus of the family is *Lyperorchis* and this is characterised by the absence of the uterine coils behind the ovary, the peculiarly drawn out and sinuous testes, the presence of two processes round the oral sucker, the absence of prepharynx and the presence of an armed cirrus.

Thus, the present form stands out clearly from all the known genera of the family.

The family Psilostomidæ appears to show variations in structures of some of the important organs in the body.   The chief modifications concern the cirrus, the position of the genital pore and the length of the uterus.   The presence or absence of the body spines is also a feature in which the genera show variations.

The cirrus is long and powerful in *Psilostomum* and *Sphæridiotrema*, and is also armed in *Lyperorchis*.   In *Psilorchis* n.g. it is the shortest.   The reduction in size of the cirrus can be traced through the series *Psilochasmus*→ *Psilotrema*→*Apopharynx*, where there is gradual reduction in size and ulti- mately we arrive at the condition found in the present genus *Psilorchis*. The cirrus pouch likewise shows corresponding reduction in size in the series.

The genital pore is situated in between the ventral sucker and the in- testinal bifurcation in *Psilostomum*.   In *Psilotrema* and *Apopharynx* it lies at the level of the pharynx, while in *Sphæridiotrema* it comes to lie at the hinder end of the mouth sucker, an extreme stage in the family.   Thus, the gradual shifting forward of the genital pore can be traced through the genera of the family.   This seems to have its effect on the length of the uterus, which is shortest in *Psilotrema* and contains only a few (4 5) eggs.   In *Apopharynx* it is a little longer and contains more eggs.   In other genera it is very long and contains many eggs.   The elongation of the uterus may also be due to the gradual shifting backward of the ovary.   In *Apopharynx* it is situated at the level of the ventral sucker, while in *Psilotrema* it is behind the ventral sucker.   In *Psilorchis* it has finally shifted much behind in the posterior half of the body.

The presence or absence of receptaculum seminis also seems to be an important feature for consideration.   It is entirely absent in the family under review, but is present in the allied family Allocreadiidæ.   Lühe (1909) mentions that the receptaculum seminis is present as a small structure in the subfamily Psilostominæ.   Odhner (1913) who revised the work of Lühe mentions its absence in the entire family, Psilostomidæ,—at least he could

not see it in any of the forms described by him. Later, Fuhrmann (1928) confirms Odhner's view in mentioning the absence of receptaculum seminis in the family under review. We have failed to find it in *Psilorchis* and some other genera in our possession. Bhalerao (1931) mentions its presence in his genus *Testifrondosa*, which he has placed under the family Psilostomidæ. It is surprising to note that he has not shown it in any of his figures. He has, however, mentioned that owing to the development of the shell glands this structure is obscured in whole mounts. If this statement of Bhalerao is accepted as correct, we fail to understand how, on the presence of the receptaculum seminis, this genus could be included under the family Psilostomidæ. We venture to suggest its removal to the family Allocreadiidæ, with which it (*Testifrondosa*) shows several other resemblances besides, *e.g.*, the position of the ovary in front of the two testes, the presence of the uterus between the ovary and the ventral sucker.

Similar variations in the structure of the cirrus, genital pore and uterus have been traced for the members of the family Allocreadiidæ, with which the present family appears to show relationships. The two families show parallel lines of evolution along divergent lines. It appears that the primitive form amongst the family Psilostomidæ shows greater resemblance to a form not much removed from *Allocreadium* and the condition in Psilostomidæ may possibly have arisen from Allocreadiidæ by the loss of the receptaculum seminis, probably through the genus *Testifrondosa*.

EXPLANATION OF FIGURES.

Fig. 1. *Psilorchis indicus* n.g., n.sp., ventral view.

Fig. 2.--*Psilorchis indicus*, showing ovary and ootype complex.

Fig. 3. *Psilorchis indicus*, showing genital pore and the cirrus.

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THE MYXOPHYCEÆ OF THE UNITED PROVINCES, INDIA.—I.

BY YAJNAVALKYA BHARADWAJA, M.Sc., PH.D. (LOND.), F.L.S.,

(From the Department of Botany, Benares Hindu University, Benares.)

ALTHOUGH the Myxophyceæ are mainly a vegetation of the tropics no serious and continued effort has yet been made to record all the forms that grow in this large country. In recent years Carter, Ghose, Brühl, Biswas and the writer have recorded a number of blue-green algæ from a few limited areas, but the number of species described is yet too small and most of the country still remains to be explored. A more vigorous and consolidated effort should therefore be made to record the myxophyceous flora of this land. The writer has been collecting these algæ for several years from various localities in the north, especially in the United Provinces, the Punjab and Kashmir, and has decided to determine them in lots. The present paper deals with twenty-one forms, representing ten genera, collected from Benares.* With the exception of *Glæocapsa atrata* (Turp.) Kütz., which was found growing on a damp wall, all the forms are aquatic. Out of twenty-one plants described below, seven are new species, three are new varieties and four are new forms.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.

I. CHROOCOCCALES.

Chroococcaceæ.

Genus *Microcystis* Kützing.

1. *Microcystis æruginosa* Kütz. Geitler, in Rabenhorst's *Kryptogamen-flora von Europa*, XIV Band, Cyanophyceæ, 1930-32, p. 136, Fig. 59d.

Colonies spherical when young, elongated and distinctly broken through when old. Sheath indistinct. Cells with pseudovacuoles.

Lat. cell., 3-4·5 μ.

Habitat :—Planktonic in a stagnant pond.

2. *Microcystis flos-aquæ* (Wittr.), Kirchn. Lemmermann, *Krypto-gamenflora d. Mark Brandenburg*, iii, Algen I, p. 75, 1910; in *Engler-Prantl Nat. Pflanzenfam.* I, 1a, p. 56, 1900.

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* Benares is situated in latitude 25° 19′ N. and longitude 83° 03″ E., at a height of 267 feet above mean sea-level. The hottest months, namely, April, May and June, have a mean maximum temperature of 113.2°F., the highest temperature recorded being 117 °F. The coldest months are December and January and they record a mean minimum temperature of 39°F., the extreme minimum temperature being 36 °F. June, July, August and September are the chief rainy months, and during this period the rainfall averages about 33″, the average annual rainfall being about 40″.

Colonies more or less rounded, unbroken, with indistinct sheath. Cells spherical, with pseudovacuoles.

Lat. cell., 3 6 μ.

Habitat :—Planktonic in a stagnant pond.

3. *Microcystis ramosa* Sp. Nov. (Fig. 1, A).

Colonies long, varying greatly in form and size, irregularly branched, constricted at intervals to form daughter-colonies which are ultimately broken off. Daughter-colonies at first almost compact and rounded, later elongated and irregularly branched like the parent. Sheath thick, unstratified, hyaline, and rather indistinct, stained violet with methylene blue. Cells spherical, with pseudovacuoles.

Lat. cell., 3 -5 μ ; diam. colon., up to 80 μ ; long. colon., up to 1,600 μ.

Habitat :—Planktonic in a stagnant pond.

This species resembles *Microcystis pseudofilamentosa* Crow in its elongated form and being composed of small colonies which break off and grow again into compound colonies, but it differs from the Ceylon species in its much larger colonies and rather smaller size of the cells. The Benares species also differs in its branched habit and being never broken through or reticulate.

Genus *Aphanocapsa* Nægeli.

4. *Aphanocapsa benaresensis* Sp. Nov. (Fig. 1, B).
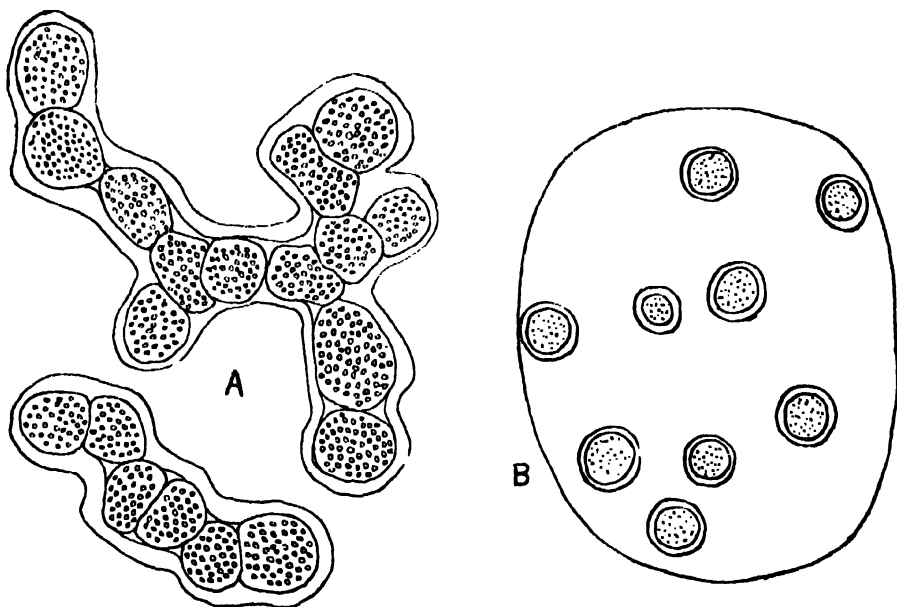
FIG. 1.

A—*Microcystis ramosa* Sp. Nov. ; B –*Aphanocapsa benaresensis* Sp. Nov.   A ×150; B ×1,200.

Plant-mass soft, spherical, hollow, irregularly broken, hyaline or cream-coloured. Cells oval or almost spherical. Sheath thick, unstratified, hyaline, closely adpressed to the cells.

Diam. plant-mass, up to 1·5 cm.; lat. cell., 4–6·2 μ; lat. vag. up to 1 μ.

Habitat:—Planktonic in a stagnant pond.

This alga approaches *Aphanocapsa Roeseana* de Bary according to the key given by Geitler (*op. cit.*), but it differs in the size and colour of the plant-masses and also in the size of the cells.

Genus *Glœocapsa* Kützing, emend. Nægeli.

5. *Glœocapsa atrata* (Turp.) Kütz. Geitler, *op. cit.*, p. 186, Fig. 83c.

Plant-mass expanded, gelatinous, slightly hard, of indefinite shape, pale-yellow. Sheath thin, hyaline, unstratified.

Diam. colon., up to 50 μ; lat. cell., 3·4 5 μ; lat. cell., cum vag., 5·2–14·5 μ.

Habitat:—On a damp wall, near a small water reservoir in the writer's house.

II. HORMOGONIALES.

(a) *Oscillatoriaceæ*.

Genus *Lyngbya* C. Agardh.

6. *Lyngbya Martensiana* Menegh. Geitler, in Pascher's *Die Süsswasser-flora Deutschlands, Österreichs und der Schweiz.*, Heft 12, *Cyanophyceæ*, 1925, p. 407, Fig. 521a.

Forma (Fig. 2, A).

Filaments single, long, straight, rarely slightly curved. Sheath thick, firm, hyaline, diffluent on the outside and exhibiting an uneven surface. Trichomes uniformly thick, without constrictions at the joints and with a row of coarse granules on either side of the indistinct cross-walls. Cells flattened; apical cell broadly rounded. Hormogones consisting of one to many cells; single-celled hormogone often getting turned over and presenting its flat surface in optical section.

Long. fil., up to 3 mm.; lat. vag., up to 3·1 μ; lat. trich., 10·5 μ; long. cell., 2·1–5·2 μ.

Habitat:—On dead leaves, among other algæ, floating in a stagnant pond.

This form agrees with the type in (1) the presence of granules by the side of the indistinct cross-walls, (2) the end-cell being broadly rounded, and (3) the colourless thick sheath; but it differs from the same in (*a*) the filaments being generally straight and not curved, and being single and never

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in bundles, (*b*) the trichomes being a little broader, and (*c*) the sheath being diffluent on the outside.
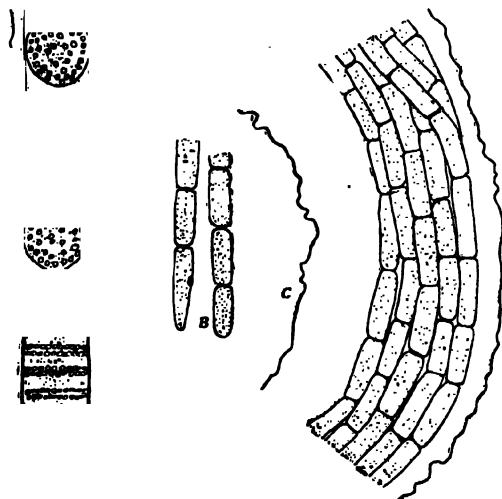
FIG. 2.

A—*Lyngbya Martensiana* Menegh. *forma* ; B—terminal portions of two trichomes and C—portion of filament of *Microcoleus chthonoplastes* Thur. All ×910.

Genus *Microcoleus* Desmazières.

7. *Microcoleus chthonoplastes* Thur. Gomont, *Monographie de Oscillariées*, 1893, Pl. XIV, Figs. 5-8. (Fig. 2, B and C).

Lat. cell., 3·1 μ ; lat. vag., up to 16·8 μ.

Habitat :—In a stagnant pond along with other algæ.

This alga differs from the type in the cells being up to four times as long as broad, and the apical cells being occasionally broadly rounded at the apex, although as a general rule they are conical.

(*b*) *Rivulariaceæ*.

Genus *Calothrix* Agardh.

8. *Calothrix scytonemicola** Tilden. Minnesota Algæ, 1910, I, p. 265, Pl. 17, Fig. 7. (Fig. 3, A and B).

Forma.

Long. fil., up to 350 μ ; lat. cell., up to 6·3 μ ; lat. heterocyst termin., 4·2-6·3 μ ; long. heterocyst subtermin., 9·4-21 μ ; lat. heterocyst subtermin., 4·2-7·3 μ.

Habitat :—On other algæ (*e.g.*, *Aulosira*) growing on floating dead leaves in a stagnant pond.

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* Geitler (*op. cit.*, 1930–32, p. 627) does not write well about this species and also about Borge's variety *brasiliensis*.

This form differs from the type in the sheath being generally distinct and the heterocysts being smaller, the terminal ones being also provided with finely granular contents. The distinctive feature in this form is the presence of basal heterocysts in pairs, one spherical or almost spherical and the other cylindrical. The other species of *Calothrix* possessing two basal heterocysts are *C. confervicola* Kg., *C. stagnalis* Gomont and *C. Castelli* Frémy with which there is no resemblance of the alga under discussion.

9. *Calothrix Ghosei* Sp. Nov. (Fig. 3, C—F).

Filaments in groups, straight or slightly bent. Sheath very distinct, thin, hyaline. Mature trichomes with slight constrictions at the distinct septa ; terminal portion not tapering into a hair and the terminal cell being
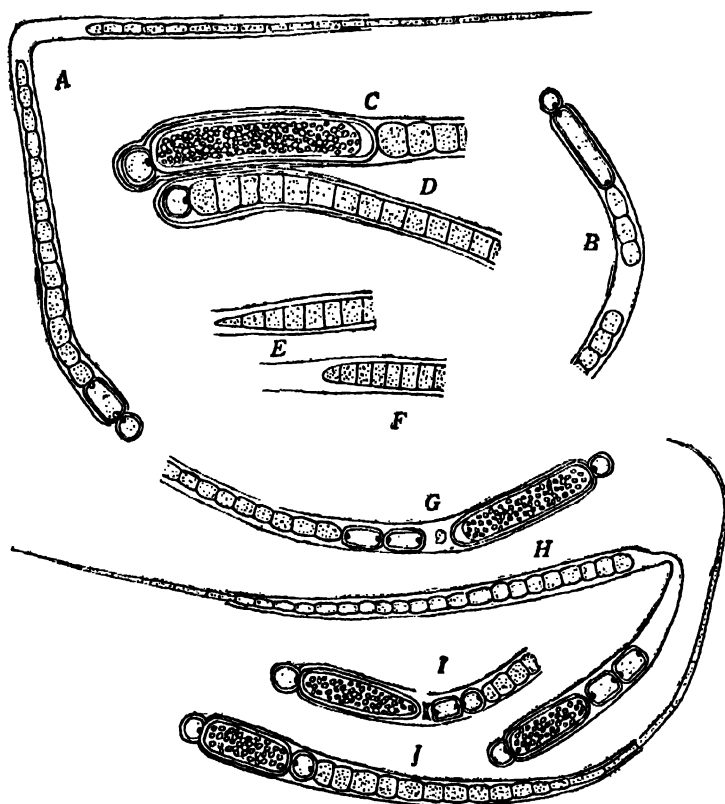
FIG. 3.

A and B—*Calothrix scytonemicola* Tilden ; C and D—basal portions and E and F—terminal portions of filaments of *Calothrix Ghosei* Sp. Nov. ; G-J—*Calothrix Fritschii* Sp. Nov.
A, B, G-J ×900 ; C-F ×640.

either sharply pointed or rounded at the apex. Cells quadratic or barrel-shaped, slightly longer or shorter than broad. Heterocysts single, basal, almost spherical or slightly broader than long, enclosed within the sheath. Spores single adjoining the basal heterocyst, cylindrical with rounded end-walls, the outer wall being smooth and hyaline.

Long. fil., up to 250 μ ; lat. fil., 7·3–13·7 μ ; lat. cell., 6·3–8·4 μ ; lat. heterocyst., 4·2–6·3 μ ; lat. spor., 10·5–12·5μ ; long. spor., 31·5–52·5 μ.

Habitat :—Epiphytic on a species of *Chara* in a stagnant pond.

This alga resembles *Calothrix gracilis* Fritsch in (1) its epiphytic habit, (2) the absence of a hair-like termination of the trichome, (3) a distinct thin sheath enclosing the basal heterocyst, and (4) the cylindrical spores ; but it differs from the same in (*a*) the filaments being shorter and broader, (*b*) the heterocysts being slightly narrower than the trichomes and being almost spherical or slightly broader than long, but never hemispherical, (*c*) the absence of intercalary heterocysts, and (*d*) the larger spores.

10. *Calothrix Fritschii* Sp. Nov. (Fig. 3, G—J).

Filaments generally in groups of 3 to 7, straight or slightly bent. Sheath very distinct, thin, hyaline, closely adpressed to the trichome. Trichomes with constrictions at the joints and tapering into a long hair, the terminal portion of which is generally unsheathed ; septa distinct. Cells barrel-shaped, as long as broad or slightly longer (or sometimes slightly shorter) than broad ; cells of the hair very much elongated and almost rectangular. Heterocysts basal and intercalary ; basal heterocysts single, spherical or sub-spherical ; intercalary heterocysts single or in pairs, spherical, quadratic or cylindrical, adjoining the basal spore or occasionally separated from it by a small dis-integrated cell. Spores single, adjoining the basal heterocyst, cylindrical or sometimes somewhat conical, with rounded end-walls ; outer wall smooth and hyaline.

Long. fil., up to 420 μ ; lat. trich., up to 6·3 μ ; lat. heterocyst., 4·2–6·3 μ ; lat. spor., 6·3–8·4 μ ; long. spor., 21–42 μ.

Habitat :—On a species of *Aulosira* growing on floating dead leaves in a stagnant pond.

The distinctive feature of this species is that, besides a single spherical basal heterocyst, there are one or two intercalary ones also adjoining the sub-basal spore. Geitler (*op. cit.*, 1930-32) has recorded only seven species of *Calothrix* which produce spores, and of these only two, *C. stagnalis* Gomont and *C. wembærensis* Hieron. et Schmidle, produce intercalary heterocysts adjoining the spores. The present species bears a striking superficial re-semblance with *C. stagnalis* Gomont in its epiphytic habit, in the trichomes ending in a long hair and in the presence of intercalary heterocysts adjoin-

ing the spores which are cylindrical or slightly conical. But it differs from Gomont's species in the much shorter filaments, the narrower and barrel-shaped cells, the presence of constrictions at the joints, the thinner spores, and in the basal heterocysts being never in pairs.

A comparison may also be made with *Calothrix wembærensis* Hieron. et Schmidle on account of the presence of barrel-shaped cells and the presence of intercalary heterocysts adjoining the cylindrical spores ; but the Benares species differs from it in the shorter filaments, a thinner sheath and narrower cells, and also in the basal heterocysts being never in pairs and the spores being thinner and never in chains.

(c)   *Scytonematacece.*

Genus *Microchæte* Thuret.

11. *Microchæte tenera* Thur. Bornet et Thuret, *Notes Alg.*, 2, Pl. XXX, Fig. 5, 1880.

Forma.

Lat. fil., 10–12 μ ; lat. trich., 8 μ ; lat. heterocyst., 8 μ ; long. hetero-cyst., 12–20 μ.

Habitat :—Along with species of *Stigeoclonium* and *Anabæna sphærica* Born. et Flah. in a stagnant pond.

The form resembles the type except for the pale brownish-purple cell-contents, the occasional slight constrictions at the joints and much broader filaments.

Var. *tenuis*, Var. Nov. (Fig. 4, A—C).

Long. fil., up to 250 μ ; lat. fil., up to 4·2 μ ; lat. trich., 3·1-3·6 μ ; lat. heterocyst., 3·6-4·2 μ.

Habitat : ·On floating dead leaves, among other algæ, in a stagnant pond.

This alga resembles the type in the thin hyaline unstratified sheath, in the cylindrical cells which are about twice as long as broad, and in the spherical heterocysts ; but it differs in the narrow filaments and trichomes, and in the absence of cylindrical intercalary heterocysts. This variety possesses the narrowest filaments out of all the freshwater species of the genus so far recorded.

12. *Microchæte grisea* Thur. Bornet et Thuret, *op. cit.*, Pl. XXX.

Var. *brevis* Var. Nov. (Fig. 4, D).

Filaments straight, at the base often curved and swollen. Sheath thin and hyaline. Trichomes with distinct septa. Cells discoid, 2-3 times as broad as long. Heterocysts single, basal, hemispherical.

FIG. 4.
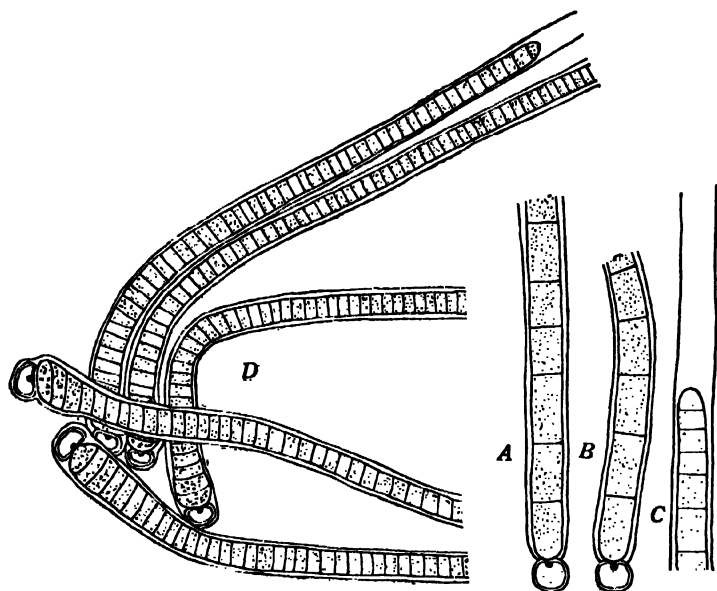
A and B—basal portions and C—terminal portion of filaments of *Microchæte tenera* Thuret,
var. *tenuis* Var. Nov.; D—*Microchæte grisea* Thuret, var. *brevis* Var. Nov.   A-C × 1370; D ×640.

Long. fil., up to 350 μ ; lat. fil., 6·8 8·3 μ ; lat. trich., 5·4 7·3 μ ; lat.
het., 6·3 10·5 μ.

Habitat :—Epiphytic on a species of *Chara* in a stagnant pond.

This form differs from the type in possessing much shorter filaments,
in the apical portion of the trichome being not widened out, and in its grow-
ing in fresh water and not in sea.

<center>(d) Nostocaceæ.</center>

<center>Genus *Nostoc* Vaucher.</center>

13. *Nostoc carneum* Ag. Geitler, *op. cit.*, 1930-32, p. 839.

Forma minor. Form. Nov.

Lat. trich., 3·1-4·2 μ ; lat. heterocyst., 4·2-5·7 μ ; long. heterocyst.,
4·2-6·3 μ ; lat. spor., 4·2-6·3 μ ; long. spor., 6·3-10·5 μ.

Habitat :—Among other algæ, in a stagnant pond.

The form resembles the type except for the smaller size of the hetero-
cysts and spores.

<center>Genus *Nodularia* Mertens.</center>

14. *Nodularia spumigena* Mertens. Bornet et Thuret, *op. cit.*, Pl.
XXIX, Figs. 10 and 11 ; Geitler, *op. cit.*, 1930–32, Fig. 554. (Fig. 5, A—C).

Lat. fil., 10–12 μ ; lat. cell., 8·4–9·4 μ ; long. cell., 2·1–3·6 μ ; lat. heterocyst., 9·4–11·5 μ ; long. spor., 5·2-7·3 μ.

Habitat :—Among other algæ in a stagnant pond.

The alga differs from the original type in the occasional development of terminal heterocysts and the smaller spores.

Genus *Anabæna* Bory.

15. *Anabæna oscillarioides* Bory. Geitler, *op. cit.*, 1930-32, Fig. 567*c*.

Var. *angustus* Var. Nov. (Fig. 5, D—F).

Trichomes single, irregularly bent or spirally coiled ; end-cell rounded. Cells barrel-shaped, as long as or slightly shorter or longer than broad. Heterocysts intercalary, very rarely terminal, ellipsoidal. Spores long cylindrical, single or in short or long chains, on both sides of the heterocysts, with smooth yellow-brown outer wall.

Lat. cell., 4·2 5·2 μ ; lat. heterocyst., 5·2–6·3 μ ; long. heterocyst., 7·3–10·5 μ ; lat. spor., 6·5–8·4 μ : long. spor., 14·7–41·0 μ.

Habitat :—Among other algæ in a stagnant pond.

This variety differs from the type in the narrower filaments, ellipsoidal heterocysts and narrower spores, which may be in long chains, with smooth yellow-brown outer wall and surrounded by a mucilaginous sheath.

16. *Anabæna sphærica* Born. et Flah., *Rev. Nost. hét., Ann. Sci. Nat. Ser.*, IV, p. 228, 1888.

Var. *tenuis* G. S. West, in Geitler, *op. cit.*, 1930-32, Fig. 560*b*.

Lat. cell., 4-5 μ ; lat. heterocyst., 4·5–6·3 μ ; lat. spor., 10-13 μ ; long. spor., 11-14 μ.

Habitat :—In a stagnant pond.

Var. *attenuata* Var. Nov. (Fig. 5, G—H.)

Thallus floccose, gelatinous, thin, free-floating, pale blue-green. Trichomes blue-green, curved or straight, more or less entangled with each other, slightly attenuated at the ends, with rounded end-cells, without a mucilage-sheath. Cells spherical or slightly barrel-shaped. Heterocysts intercalary, spherical or slightly pressed from both sides. Spores single, on one or both sides of the heterocyst, spherical or oval, with smooth and yellow-brown outer wall.

Lat. cell., 3·2–5·2 μ; lat. heterocyst., 5·2–6·2 μ; lat. spor., 10·5–12·6 μ; long. spor., 10·5–14·7 μ.

Habitat :—In a stagnant pond.

The alga resembles the type in the spherical or barrel-shaped cells, spherical or oval heterocysts, and single, spherical or oval spores on one or both sides of the heterocysts. It comes close to var. *tenuis* G. S. West on
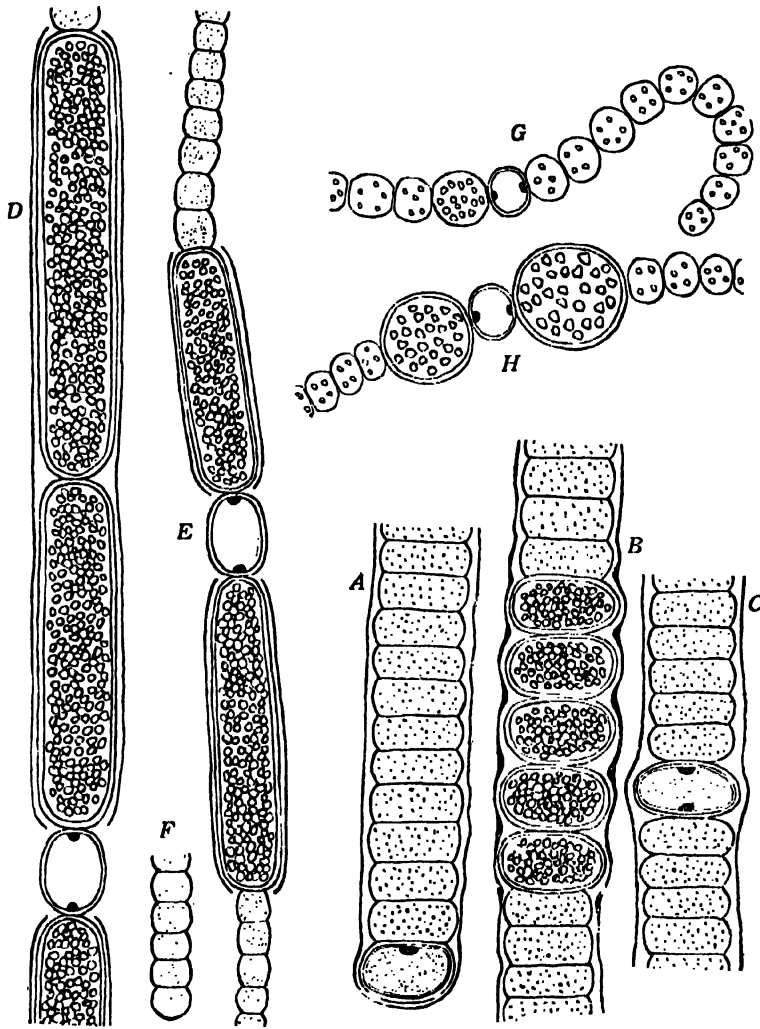
FIG. 5.

A-C—*Nodularia spumigena* Mertens.; D and E –sporogenous portions and F—terminal portion
of filaments of *Anabæna oscillarioides* Bory, Var. *angustus* Var. Nov.; G and H—*Anabæna
sphærica* Born. et Flah., Var. *attenuata* Var. Nov.   All ×1370.

account of the narrow trichomes, and smaller heterocysts and spores, but
it differs from this variety and the type in the thallus being pale blue-green,
in the trichomes being curved in various ways and more or less entangled
with each other and being slightly attenuated at the ends, in the spores being
never in pairs, and in the slight variations in the dimensions of all parts.

From the variety *tenuis* G. S. West it further differs on account of the presence of spherical cells and the heterocysts being slightly pressed from both sides.

17. *Anabæna doliolum* Sp. Nov. (Fig. 6, A and B.)

Plant-mass mucilaginous, pale blue-green. Trichomes single; free swimming; straight, curved or slightly coiled; slightly tapering at the ends; with conical apical cell, possessing almost pointed apex. Cells barrel-shaped as long as broad or a little longer or shorter than broad. Heterocysts barrel-shaped. Spores ellipsoidal with almost pointed apices in short or long chains adjoining the heterocysts but developed centrifugally, with thick, smooth, and hyaline or yellow-brown outer wall.

Lat. cell, $3 \cdot 6 - 4 \cdot 2 \mu$; lat. heterocyst., $5 \cdot 2 - 6 \cdot 3 \mu$; long. heterocyst., $6 \cdot 3 - 9 \cdot 4 \mu$; lat. spor., $4 \cdot 2 - 6 \cdot 2 \mu$; long. spor., $6 \cdot 3 - 11 \cdot 5 \mu$.

Habitat :—Among other algæ in a stagnant pond.

This alga is quite unique and differs from all other species in the possession of barrel-shaped cells and heterocysts, and chains of ellipsoidal spores, with almost pointed ends, adjoining heterocysts but developed centrifugally.

18. *Anabæna kashiensis* Sp. Nov. (Fig. 6, C—G.)

Thallus dense, soft, mucilaginous, deep green. Trichomes blue-green; often irregularly curved and more or less entangled with each other, slightly constricted at the joints, attenuated at the ends; the terminal cell being often conical with a sharp or rounded apex; without mucilage-sheath. Cells cylindrical, up to twice as long as broad, rarely barrel-shaped and almost as long as broad. Heterocysts single, intercalary and distributed at regular intervals throughout the length of the trichome, cylindrical. Spores in short or long chains, ellipsoidal or barrel-shaped, remote from the heterocysts; with outer wall thick, smooth and colourless.

Lat. cell., $3 \cdot 1 - 4 \cdot 2 \mu$; lat. heterocyst., $4 \cdot 2 - 5 \cdot 2 \mu$; long. heterocyst., $8 \cdot 4 - 12 \cdot 6 \mu$; lat. spor., $4 \cdot 2 - 6 \cdot 3 \mu$; long. spor., $6 \cdot 3 - 10 \cdot 5 \mu$.

Habitat :—On floating dead leaves in a stagnant pond.

The alga approaches *Anabæna variabiles* Kütz. in the deep green mucilaginous thallus, the presence of slight constrictions at the joints of the trichome, the conical end-cells, and in the barrel-shaped spores formed in chains remote from the heterocysts. It, however, differs in the elongated cylindrical cells and heterocysts and in the smaller breadth of the trichome, heterocysts and spores.

19. *Anabæna Iyengari* Sp. Nov. (Fig. 6, H—K.)

Trichomes single, straight or irregularly curved; end-cell conical with rounded apex. Cells barrel-shaped, as long as broad or slightly shorter or longer than broad. Heterocysts barrel-shaped, rarely spherical. Spores
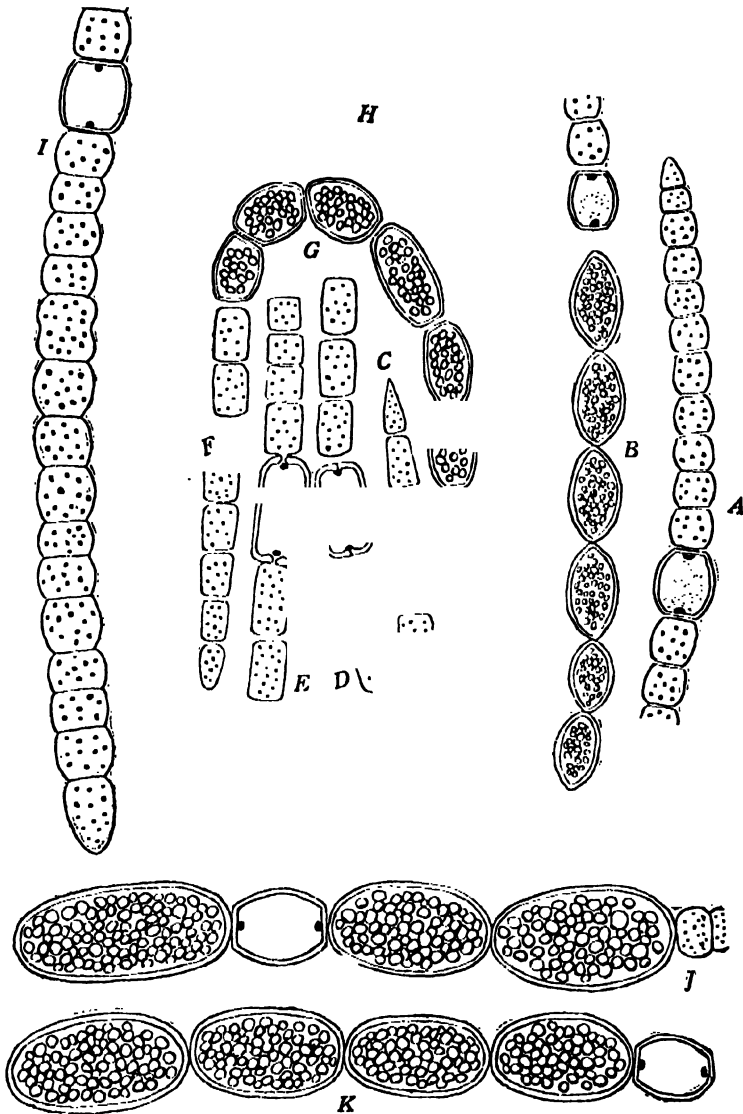
FIG. 6.

A and B—*Anabæna doliolum* Sp. Nov.; C-G—*Anabæna kashiensis* Sp. Nov.;
H-K—*Anabæna Iyengari* Sp. Nov.   All ×1370.

ellipsoidal, often in long or short chains, rarely single, on both sides of the
heterocysts, with thick, smooth, yellow-brown outer wall.

Lat. cell, $5 \cdot 2$–$6 \cdot 3 \mu$; lat. heterocyst., $7 \cdot 3$–$8 \cdot 4 \mu$; long. heterocyst., $7 \cdot 3$–$10 \cdot 5 \mu$; lat. spor., $8 \cdot 4$–$10 \cdot 5 \mu$; long. spor., $10 \cdot 5$–$21 \cdot 0 \mu$.

Habitat :—Among other algæ in a stagnant pond.

This alga can only be compared with *Anabæna sphærica* Born. et Flah. ; both have barrel-shaped cells, and the spores are developed on both sides of the heterocysts. The present species, however, differs in the presence of conical end-cells of the trichomes, the larger barrel-shaped heterocysts, and the larger ellipsoidal spores formed often in long or short chains.

A greater part of this work was done in the Botanical Laboratory of the East London College (now Queen Mary College), and the writer expresses his grateful thanks to Professor F. E. Fritsch, F.R.S., for his kind help.

INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part X. Influence of Different Forms of Manganese on the Oxidation of Organic Matter and Release of Plant Nutrients.

By C. R. HARIHARA IYER, M.A., R. RAJAGOPALAN, B.Sc. (HONS.),

AND

V. SUBRAHMANYAN, D.Sc., F.I.C.

(*Department of Biochemistry, Indian Institute of Science, Bangalore.*)

IT was shown in the previous communications (Subrahmanyan and Siddappa, 1933 ; Harihara Iyer, Rajagopalan and Subrahmanyan, 1934 ; Siddappa and Subrahmanyan, 1935) that small amounts of oxidising agents such as potassium permanganate, hydrogen peroxide, ferric oxide or manganese dioxide, when applied to soil, can improve the growth of plants and increase the yield of crop. Evidence was also adduced to show that such chemicals hasten the oxidation of organic matter and increase the production of carbon dioxide. In the case of manganese dioxide there was a momentary increase in the numbers of bacteria, actinomyces and fungi but subsequent effect was not appreciable. It was not clear, however, as to how far the beneficial effects were due to the bases with which the different chemicals were associated ; as to how much was due to purely ionic effects regarding which there is an extensive literature (Brenchley, 1927 ; Harihara Iyer *et al., loc. cit.*) and how much to oxidation. The mechanism of oxidation is still obscure. Further work is needed to show how far the changes are due to purely chemical action and, to what extent, they are brought about by micro-organisms. Fresh information is also required regarding the comparative merits of the different chemicals used for the purpose and their behaviour under diverse soil conditions. In view of the above and the need for further knowledge regarding the application of oxidising agents as fertilisers, the present enquiry was undertaken. Since the previous study has shown that potassium permanganate and manganese dioxide were highly potent in their action, the immediate research was confined to compounds of manganese.

Experimental.

Plot experiments with ragi (Eleucine coracana).—The trials were carried out on a number of small plots, each measuring 121 sq. ft. in area. The

following were the treatments :—(*a*) Unmanured (control) ; (*b*) manured at 15 lbs. per plot (hongay cake) ; (*c*) unmanured and treated with potassium permanganate (150 g. per plot) ; (*d*) manured and treated simultaneously with permanganate; and (*e*) same as (*d*) but with the permanganate applied as top dressing in two instalments. Four plots were allotted for each treatment except (*b*) which received eight. They were distributed according to the random method.

A week after the application of manure, the seeds (H_{22} variety) were sown (12–6 1934). Germination was quite satisfactory and on 6th July, the seedlings were thinned out to 200 per plot. The first instalment of top dressing was given on 6th August and the second on 13th. Owing to scarcity of rainfall, the plots had to be irrigated with tap water. The growth was, nevertheless, quite luxuriant, especially on the manured plots and the plants were ready for harvest towards the end of October. The grain and straw representing each treatment were dried separately and their weights determined.

In the previous season (1933), similar treatments had been tried on the same plots (Harihara Iyer, *et al.*, *loc. cit.*). The only important difference was that farmyard manure was used in place of the cake and was applied at 22 lbs. per plot (approximately 3 tons per acre). Since the object of that experiment was the same as that of the present one, the two sets of results have been presented together in Table I. The yields have been given as from 484 sq. ft. (1/90th of an acre) in each case.

TABLE I.

| Treatment | Yield (dry wt.) in Kg. | | | |
| | Fy. Manure (1933) | | Hongay Cake (1934) | |
	Grain	Straw	Grain	Straw
Untreated (Control)	1·13	1·55	5·53	15·67
Manure alone	2·18	2·10	14·86	47·51
Permanganate alone	2·07	1·79	8·30	15·71
Manure+Permanganate (simultaneously)	2·87	2·80	18·79	52·00
Manure+Permanganate (top-dressing)	2·31	2·31	16·27	49·93

The meteorological observations for 1933 have been discussed in the previous communication. Analysis of the data* for 1934 would show that the season (July-October) was moderately warm, the maximum temperature ranging from 75-90°F. The nights were comparatively mild, the minimum temperature rarely going below 65°F. As compared with the previous season, rainfall was generally meagre except during the middle of October when there were about 5 inches of rain in the course of one week. There were light showers (generally a few cents at a time) both at the beginning and at the end of July. The showers were more frequent during August, but September (except for a few days in the middle) was comparatively dry. By about the middle of October, the crops had come to harvest, so they derived no benefit from the heavy rains which followed. Relative humidity was generally lower than in the previous years and ranged between 50 and 70 per cents. except for short periods both during and after the showers. The season being comparatively dry, the plots had to be regularly watered almost upto the time of harvest.

It may be noted that, in both the seasons, the yields from the manured as well as unmanured plots were increased as the result of application of permanganate. Of the two methods of application, simultaneous appli-cation with the manure yielded better results than top-dressing in instal-ments.

Although the cake was richer in fertilising ingredients (N, 4·5 per cent.) than farmyard manure (N, 0·8 per cent.), it may yet be observed that the comparatively heavy yield of 1934 was not entirely due to manurial treat-ment. Even the unmanured (control) plots yielded about four times as much grain and ten times as much straw in 1934 as in 1933. The ratio of grain to straw was approximately as 1 : 1 in 1933 and about 1 : 3 in 1934. The latter observation applies to the yields from all the treatments in each season. It would thus be seen that while the mode of action remains the same, the extent of benefit derived from application of the oxidiser may be greatly modified by the season.

Distribution of manganese in the experimental plots.—Some preliminary observations made shortly after the different treatments showed that there was no free permanganate in any of the experimental plots. This observation suggested that the permanganate was rapidly reduced by the organic matter of the soil. With a view to determining the mode of interaction between permanganate and the soil, the distribution of manganese in the different

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* Obtained through the courtesy of Mr. C. Seshachar, Meteorologist to the Government of Mysore, to whom the authors' thanks are due.

experimental plots was studied. Representative specimens (10 g.) of air-dried soil were first extracted with water and the quantities of manganese present in the extracts determined. The residual soil was then extracted with 4 N sulphuric acid and the manganese in the extract estimated as before. Fresh specimens (10 g.) of soil were then treated with sodium sulphite (5 g.) and sulphuric acid (4 N, 100 c.c.) and the total manganese brought into solution on boiling the mixture was estimated. The last procedure was found to be the most suitable for the extraction of total manganese including resistant forms such as manganese dioxide. A number of preliminary trials showed that the extraction was rapid and quantitative. Only small quantities of ferrous iron and other interfering substances were present in solution. Boiling removed the excess of sulphur dioxide.

The estimations of manganese in solution were carried out according to the bismuthate method. Some difficulty was experienced on account of the presence of chlorides in the soil, as also in certain commercial preparations of sodium bismuthate. This was overcome, however, by adding small quantities of mercuric oxide or sulphate (2-3 g.) to the hot soil-bismuthate suspension, just prior to filtration, for estimation. There was not even a trace of chloride in the filtrate. Silver salts were not useful for this purpose, as they formed soluble compounds which interfered with the estimation. Moreover, heating with silver salts (*e.g.*, the sulphate) caused the rapid decomposition of the bismuthate so that there was none left to oxidise the manganese to the permanganate condition. Attention may also be drawn to the small correction to be applied for the oxidising action of water-soluble matter from the bismuthate itself. A simple method embodying the foregoing principles and specially designed for the estimation of manganese in soils and biological media has been developed and will be described in a later communication.

The results have been presented in Table II.

The original soil itself contained fairly large quantities of manganese which was present partly in the acid soluble and partly in the insoluble condition. The added permanganate contributed about a ninth of the original manganese. About half of it passed into the acid soluble condition and the other half into insoluble forms. Since the reaction between soil and permanganate proceeded very rapidly it would appear that the increased yields were largely due to the acid soluble and insoluble products resulting from the treatment.

Effect of Applying Different Forms of Manganese to Soil.

Distribution of manganese.—Before conducting a systematic study of the effect of different forms of manganese on organic matter, it was considered

TABLE II.

Treatment to original soil	Manganese in parts per million		
	Water soluble	Acid soluble	Total
Unmanured (Control)	Traces	89·5	360·8
Manured with hongay cake	Do.	85·8	371·8
Unmanured + KMnO₄	Do.	118·8	398·0
Manured + KMnO₄ (Simultaneous)	Do.	107·8	393·6
„ + „ (Instalments)	Do.	118·8	387·2

desirable to obtain an idea of the changes that attend their application to the soil. A number of pots were made up with soil-sand mixture (30 lbs. ; proportion, 3 : 1). They were then treated with finely powdered hongay cake (30 g.) and divided into five batches. One set of pots was treated immediately with potassium permanganate at the rate of 4·5 g. per pot. The others were rested for three days and then treated as follows:—(*a*) manganous sulphate at 4·3 g. per pot ; (*b*) manganese dioxide (7·5 g.) ; (*c*) manganous carbonate (10 g.) ; and (*d*) control (untreated). The quantity of manganous sulphate was equivalent to that of permanganate on the manganese basis and that of manganous carbonate to that of manganese dioxide. Cork-borer samples of soil were taken from the pots at stated intervals and the distribution of manganese determined in the manner outlined already. The results were as follows (Tables III A and III B).

TABLE III A.

Treatment (Samples taken immediately after treatment)	Manganese in parts per million		
	Water soluble	Acid soluble	Total
Soil + Hongay Cake (Control)	Traces	135·5	453·9
„ + „ + KMnO₄	Do.	147·8	569·4

It may be noted that, with the exception of manganous sulphate, none of the treatments yielded any water soluble manganese. Even in the former case, more than two-thirds was present in insoluble forms. The water

TABLE III B.

| Form of Manganese applied | Manganese in parts per million | | | | | |
| | After 1 hour | | | After 24 hours | | |
	Water soluble	Acid soluble	Total	Water soluble	Acid soluble	Total
MnSO₄	32·3	170·8	544·8	18·5	154·0	537·0
KMnO₄	Traces	150·8	540·1	Traces	143·1	563·2
MnO₂	Do.	137·0	849·4	Do.	132·3	864·8
MnCO₃	Do.	635·6	854·1	Do.	587·9	843·3
Untreated (Control) ..	Do.	126·2	381·7	Do.	127·7	386·3

soluble manganese tended to steadily diminish and after a week, there was none left in any of the pots. Acid soluble forms which represented a useful proportion of the total manganese in all the cases would include the carbonate, the phosphate and such other compounds. The estimate should also include any manganese that might be present as exchangeable base or otherwise retained by the mineral complex of the soil system. The original soil itself contained fairly large quantities of such forms and there were further additions through the different treatments—especially manganous sulphate and carbonate. In the latter case, the carbonate itself would have contributed to the increase in acid soluble forms. The acid insoluble forms would represent the major part of the manganese in the soil. In two of the cases they were present mostly as manganese dioxide, whereas in the others, especially that of treatment with manganous sulphate, the position is still obscure. Further systematic research is needed to throw light on the mode of interaction between soil and different forms of manganese and its bearing on the various chemical and biological transformations in the soil.

The immediate effect of some of the soluble forms of manganese on soil bacteria.—It has already been shown (Harihara Iyer, *et al., loc. cit.*) that in both the manured and unmanured soil, application of manganese dioxide causes a momentary increase in the number of bacteria and actinomyces.

With a view to determining the effect of applying the other forms of manganese the following experiment was carried out.   To 10 g. lots of soil, powdered seed-cake (0·2 g.) was added and the mixtures treated as follows:—(a) Manganous sulphate (0·43 g.) ; (b) potassium permanganate (0·45 g.) ; and (c) control (untreated).   They were then moistened with water (4 c.c.) and left at the laboratory temperature (25-28°C.) so as to simulate the field   condition. Samples were taken out at intervals and plated on Thornton' smedium (1922). The bacterial counts were as follows (Table IV).

TABLE IV.

Treatment	Bacterial numbers per gram of soil		
	At the commencement	4th day	10th day
MnSO$_4$	92 × 10^4	10 × 10^5	50 × 10^6
KMnO$_4$	1.6 × 10^3	15 × 10^5	60 × 10^6
Untreated (Control)	25 × 10^6	120 × 10^6	135 × 10^6

Unlike manganese dioxide, the two soluble forms caused an immediate depression in the number of bacteria.   Thus, in the case of samples treated with permanganate, the numbers at the commencement were less than a thousandth of those in the control.   Later on, there was steady recovery and on the 10th day, the numbers were just under half of those in the untreated samples.   Although the quantities of permanganate and manganous sulphate added in the above experiment were rather large, it should nevertheless be admitted that those two forms act as partial sterilisers and that bacterial action would, at any rate momentarily, be retarded.   Further work is needed to throw light on the significance of the above in relation to field practice.   In the case of permanganate it has already been observed (Harihara Iyer, et al., loc. cit.) that the maximum benefit can be obtained only when it is applied together with the manure, a few weeks before sowing or transplanting as the case may be.   Whether similar observations would apply to manganous sulphate still remains to be determined.

Decomposition of organic matter in presence of different forms of manganese. —It has already been shown that treatment with oxidiser is attended by fairly rapid loss of carbon (Harihara Iyer, *et al., loc. cit.*).   With a view to determining whether (a) the changes follow a similar course in presence of

different forms of manganese and (*b*) the extent to which purely chemical action accounts for the various transformations, the following experiments were carried out. The soil (red sandy loam similar to that used for the various pot and plot experiments) was weighed out in 10 g. lots into a number of Petri dishes and treated with finely powdered hongay cake (0·2 g.). The dishes were then divided into five different batches and treated as follows :—
(*a*) manganous sulphate (0·43 g.) ; (*b*) potassium permanganate (0·45 g.) ; (*c*) manganese dioxide (0·75 g.) ; (*d*) manganous carbonate (1·0 g.) ; and (*e*) untreated (control). Half the number of dishes receiving each treatment were left as such while the others were sterilised by autoclaving for 1 hr. at 20 lbs. followed by dry heating at 110° for 1 hrs. The heating was not carried out at a still higher temperature because it was feared that such a treatment might seriously alter the composition of the soil. The heated samples were moistened with sterile water (1 c.c.) and the others with distilled water. The dishes covered with lids were then spread out on a number of tables, in positions where they received a portion of the day's sunlight. This was done with the object of simulating, as far as possible, the conditions prevailing in the experimental pots. At stated intervals, the entire contents of representative specimens were washed down with minimum quantity of water, into digestion flasks and their carbon contents estimated according to Subrahmanyan, Narayanayya and Bhagvat (1934). The results have been presented in Figs. 1 and 2.

Total carbon in soil, 0·13 per cent.; in cake, 35·1 per cent.; total quantity of carbon at the beginning in the control and in the samples treated with different forms of manganese except the carbonate, 113·2 mg. each ; in samples treated with manganous carbonate, 198·6 mg. each.

It may be noted that in both the sets, the maximum loss of carbon occurred in the case of specimens treated with potassium permanganate or manganese dioxide. The latter was slightly more, but the difference was not considerable. Manganous carbonate came next, but it is difficult to state as to what part of the carbonate itself was decomposed in the process. Manganous sulphate followed later and was not very different from the control, which came last.

A comparative study of the two sets of results would show that microbial activity would account for the major part of the loss of carbon. There is nevertheless evidence of considerable amount of decomposition through purely chemical action. The latter effect is most pronounced in the case of specimens treated with the permanganate or manganese dioxide. This would show clearly that chemical oxidisers can act independently of micro-organisms and bring about the decomposition of organic matter. Even the
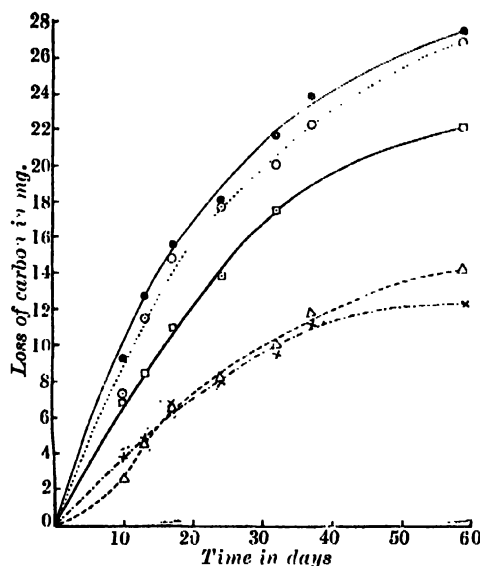
FIG. 1.　Non-sterile (ordinary) soil.　　　　FIG. 2.　Sterile soil.
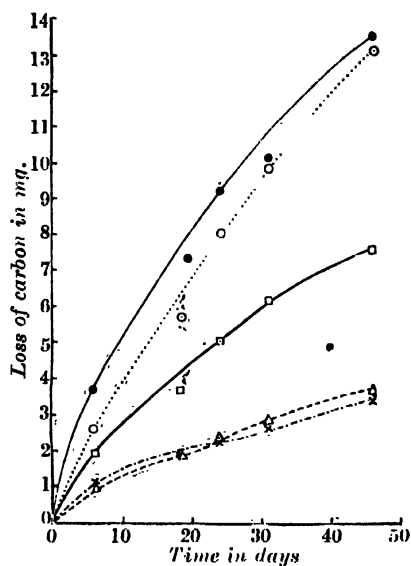
✕—✕ Control　　△---△ MnSO₄　　⊙---⊙ KMnO₄　　•—• MnO₂　　◻—◻ MnCO₃

FIGS. 1 and 2.　Loss of carbon consequent on the application of different forms of manganese.

controls (untreated samples) showed some loss of carbon. This is to be expected when considering that the original soil itself contained useful quantities of oxidative minerals such as ferric oxide (Harihara Iyer, *et al.*, *loc. cit.*).

It would thus be seen that there were at least three agencies concerned in the decomposition of organic matter—the mineral matter of the soil, the added chemical and the micro-organisms. The first and the third would depend largely on the nature of the soil and the conditions prevalent in it. The chemical action would be partly determined by the composition of the soil. The added chemicals would, in turn, influence the activity of micro-organisms. Allowing for the above, it should still be possible to obtain estimates of the decomposition brought about, directly or otherwise, through the agency of the added chemicals. The results thus obtained have been presented in Table V.

It may be noted that there was generally greater loss of carbon from non-sterile samples than from the corresponding sterile ones. The available evidence is not sufficient to explain this observation, but it is probable that the chemical oxidation was facilitated by microbial activity which resulted in a preliminary disintegration of organic matter.

TABLE V.

Decomposition of Organic Matter through purely Chemical Oxidation.

Form of Manganese	Carbon lost (in mg.) at the end of							
	10 days		20 days		30 days		40 days	
	Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
Manganous sulphate ..	0·2	0·3	..	..	0·3	0·5	0·3	0·8
Manganous carbonate ..	1·4	3·4	2·4	5·5	3·5	7·5	4·0	8·5
Potassium permanganate ..	2·5	5·4	4·9	9·3	7·1	10·4	8·8	11·9
Manganese dioxide ..	3·8	5·8	6·1	10·3	8·2	11·9	9·6	13·5

Among the different treatments, that with manganese dioxide was the most effective under both sterile and non-sterile conditions. Permanganate came a close second. Its action followed very nearly the same course as that of manganese dioxide. This was due to the rapid decomposition of permanganate resulting in the formation of manganese dioxide. There was fairly rapid loss of carbon from samples treated with manganous carbonate, but since no separate determinations of the residual carbonate were carried out, it is difficult to state as to what part of the loss was due to organic carbon. Manganous sulphate came last and was in fact no better than the untreated control.

Production of carbon dioxide.— The treatments were the same as those in the previous experiment. The only difference was that ten times the previous quantities were taken. The samples were kept in Erlenmeyer flasks (cap. 1 litre) fitted with two-holed rubber stoppers. Carbon dioxide was estimated by displacement with (CO_2 free) air followed by absorption in excess of alkali, a Truog trap (1918) being used for the purpose. The results have been presented in Figs. 3 and 4.

Production of carbon dioxide followed nearly the same course as loss of carbon. As in the previous series, manganese dioxide was the most active with permanganate following as a close second. Manganous carbonate was the third, while manganous sulphate was just better than the control. As
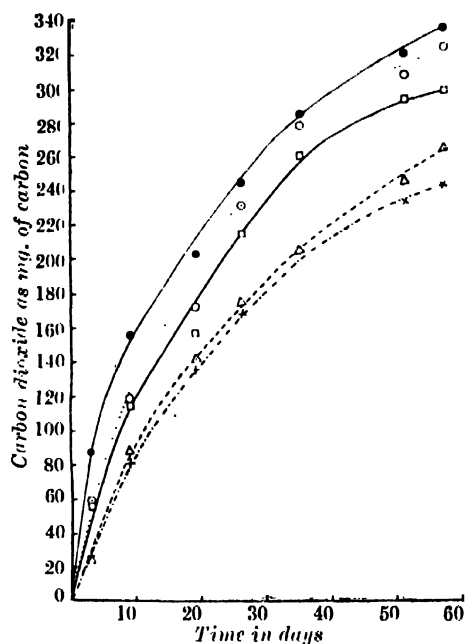
FIG   Non-sterile (ordinary) soil.   FIG. 1. Sterile soil.

Control   ▵--▵ MnSO₄   ◉---◉ KMnO₄   ●--● MnO₂   ◻--◻ Mn(O₃
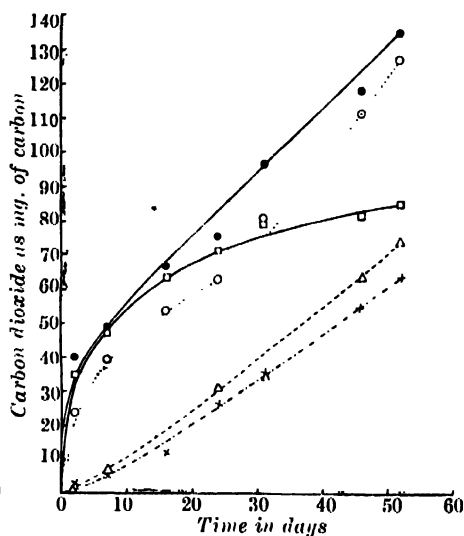
Figs. 3 and 4. Production of carbon dioxide on treatment with different forms
of manganese.

may be naturally expected, the major part of the gas produced from the non-
sterile soil was through biological activity. A useful proportion was formed
through purely chemical action with the original minerals of the soil. Allowing
for these, the quantities of carbon dioxide formed through the agency (direct
or otherwise) of the added chemicals may be estimated as follows (Table VI).
As in the previous series, the chemical action was more pronounced in the
case of the non-sterile samples than in those of the sterile ones. Since the
loss of carbon and production of carbon dioxide are inter-related, it may
be assumed that the changes indicated in an earlier section would apply to
the present series as well.

It would be difficult to state whether all the carbon dioxide was derived
from the added manure. It may be reasonably expected, however, that the
major part came from that source, because the manure was readily ferment-
able and contained more of available carbon and nitrogen than the organic
matter of the soil. The quantities of carbon affected by the different treat-
ments varied considerably, but even under the most favourable conditions

TABLE VI.

Form of Manganese	CO$_2$ produced (as mg. of c) at the end of							
	10 days		20 days		30 days		40 days	
	Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
Manganous sulphate	2	5	7	..	7	5	7	5
Manganous carbonate	49	32	50	40	..	50	..	61
Potassium permanganate	41	39	41	57	41	70	47	78
Manganese dioxide	51	59	59	69	61	79	66	86

(such as that provided by manganese dioxide) did they exceed half the amount of added organic matter.

The extents to which the different agencies contributed to the oxidation also varied with the treatment, but it may be mentioned that, in all the cases, the micro-organisms were the most active. Thus, chemical treatment was next in prominence in two of the cases (manganese dioxide and permanganate), while in the other two, its effect was not so pronounced. Oxidation by the original minerals of the soil (which was common to all the samples) would account for about a fourth of that brought about by microbial action. Assuming that the other two agencies functioned in the same manner, it would be seen that chemical treatment contributed substantially to the oxidation of organic matter in the soil.

The foregoing observations relate only to conditions prevalent in the absence of the growing plant. It is well known that the presence of vegetation hastens the oxidation of organic matter (Neller, 1922 ; Siddappa and Subrahmanyan, *loc. cit.*). The penetration of roots increases the air space in the soil and facilitates quicker absorption of oxygen. The production of carbon dioxide would also be augmented by plant respiration. It would be of much practical interest to investigate the action of chemical oxidisers under such conditions.

It would be rather difficult to trace a quantitative relation between the loss of carbon and production of carbon dioxide in the two foregoing sets of

experiments. The containers were of different types. The procedure for the estimation of carbon dioxide involved increased supply of air to the samples used for that study. It would nevertheless be seen (Figs. 5 and 6) that there is close positive correlation between the loss of carbon on the one hand and production of carbon dioxide on the other. The percentages have been calculated on the basis of the total carbon originally present in the soil system.
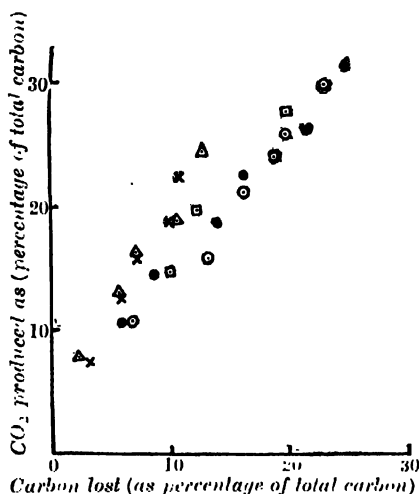
FIG. 5. Non-sterile (ordinary) soil.   FIG. 6. Sterile soil.
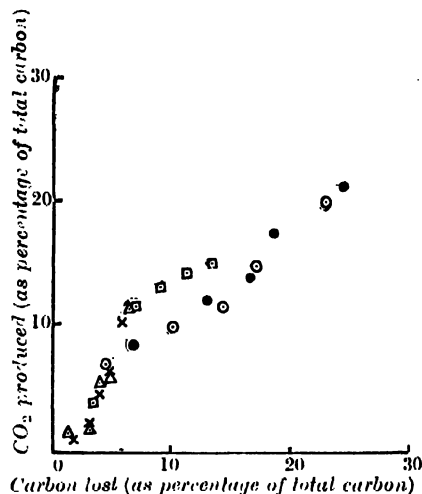
× Control   ▲ MnSO₄   ◎ KMnO₄   ● MnO₂   ▣ MnCO₃

FIGS. 5 and 6. Diagrams showing the correlation between loss of carbon and production of carbon dioxide in samples treated with different forms of manganese.

Although the foregoing observations would point to carbon dioxide as the chief product of chemical as well as biological action, it is nevertheless possible that some of the intermediate products are also of much physiological importance. This inference is further supported by the observation that the increased production of carbon dioxide consequent on the application of chemical oxidisers does entirely account for the greatly improved yield observed in the case of many crops. Similar observations would also apply to nitrogenous compounds. Although there is no perceptible change in total nitrogen (Harihara Iyer, *et al.*, *loc. cit.*) it is yet possible that some of the degradation products of the proteins of the cake may be readily available to plant nutrition.

Effect of application of different forms of manganese on the yield of tomato.— In the previous study (Harihara Iyer, *et al.*, *loc. cit.*) it was observed that tomato responded best to application of manganese dioxide. The observations

showed that the dioxide greatly increased the availability of organic manure ; but it was not clear, however, as to whether the beneficial effect was entirely due to oxidation of organic matter. It was considered prob- able that the response might change with the variety of tomato. The nature of the soil and particularly its reaction might also influence the yield. With a view to obtaining some information regarding the above, the follow- ing experiments were carried out. Over a thousand pots were made up with soil-sand mixture (30 lbs. ; proportion, 3 : 1) in the usual way. Half the number of pots were treated with burnt lime at 15 g. each. After a week's rest, all the pots were treated with superphosphate (non-acid, con- centrated) at 3·0 g. each. This was followed by application of powdered hongay cake (N, 4·5 per cent.) at 30 g. per pot. A week later, the seeds were sown, 100 pots being allotted for each variety. Six to eight seeds were sown per pot, but as the seedlings came up, they were reduced to two each. A fortnight after germination, all the pots were top-dressed with potassium nitrate (2·0 g. each) and potassium sulphate (1·5 g. each) respectively. A week later, the pots were divided into groups of five and treated as follows : (a) manganous sulphate at 4·3 g. per pot ; (b) manganese dioxide at 7·5 g. ; (c) manganese carbonate at 10 g. ; and (d) left untreated (control). Another set of pots (20 for each variety) was made up similarly, but treated with potassium permanganate (1·5 g. per pot) immediately after application of the cake. Those pots were sown at the same time as the previous series and received similar top dressings of nitrate and potash. In addition to the above, a further control series was started in which all the treatments were the same as those obtained above except that the organic manure was not applied. The following eight varieties were tried :- (1) Ponderosa ; (2) Bonnie Best ; (3) Globe ; (4) Marglobe ; (5) Perfection ; (6) King Hum- bert ; (7) Cherry Red , and (8) Golden Jubilee. Twelve pots of each variety were allotted for each form of manganese. Of these, six were limed while the other six were unlimed. A similar number was allotted for unmanured controls.

The seeds were sown on 13th August 1934. Germination was gene- rally satisfactory, but the seedlings which came up in the limed pots began dying rapidly, so that they had to be mostly resown. The seedlings were thinned out on 11th September and the top-dressings applied on the 13th and 14th. Flowering began on the 18th September and fruit production from the 13th October. Fruiting stopped towards the middle of January, the season being rather short, as compared with those prevailing in the temperate regions.

The fruits representing the different treatments, as well as varieties, were collected from time to time and their fresh weights determined. The

yields thus obtained at each stage have been presented in Figs. 7 22. The average yields for each treatment have been presented in Tables VII and VIII.

TABLE VII.

Effect of different forms of manganese on the yield of tomato.

Variety	Average yield per plant (in g.) on treatment with				
	$MnSO_4$	$KMnO_4$	MnO_2	$MnCO_3$	Untreated (control)
Ponderosa	355	418	439	301	286
Bonnie Best	257	271	264	248	247
Globe	288	310	335	256	258
Marglobe	248	274	311	238	214
Perfection	211	292	315	176	208
King Humbert	325	450	424	282	282
Cherry Red	217	301	341	141	170
Golden Jubilee	257	380	435	232	220

TABLE VIII.

Effect of lime on the response of tomato to different forms of manganese.

Variety	Average yield per plant (in g.) on treatment with				
	$MnSO_4$	$KMnO_4$	MnO_2	$MnCO_3$	Untreated (control)
Ponderosa	202	213	225	187	125
Bonnie Best	192	223	229	151	141
Globe	122	192	150	105	87
Marglobe	211	233	224	110	114
Perfection	194	265	270	158	132
King Humbert	146	248	208	134	140
Cherry Red	250	298	357	176	223
Golden Jubilee	111	164	188	101	84
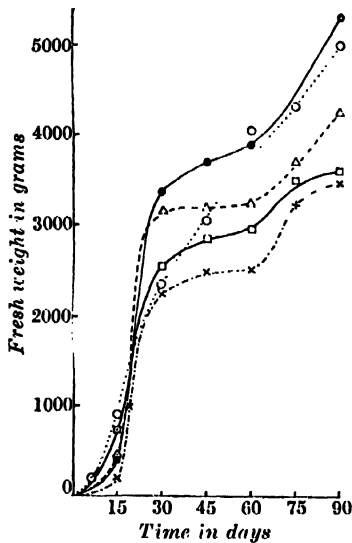
FIG. 7. Variety—Ponderosa.
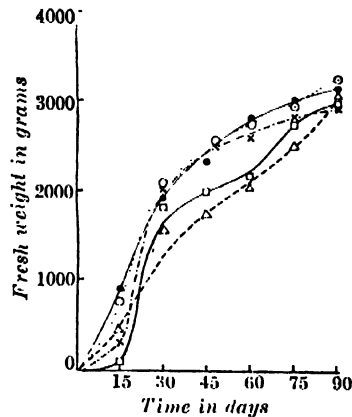
FIG. 8. Variety—Bonnie Best.
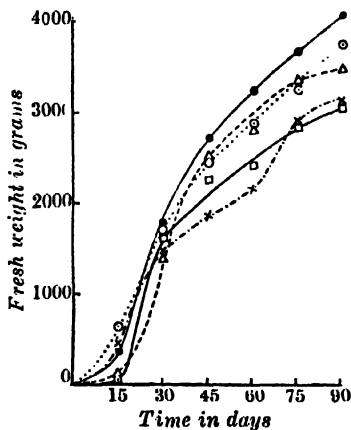
FIG. 9. Variety—Globe.
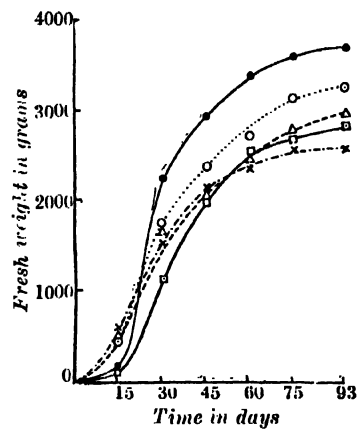
FIG. 10. Variety—Marglobe.

The unlimed plants came out generally better than the limed ones and yielded more fruit. Among the different treatments, permanganate and manganese dioxide were the most effective. It is difficult, however, to decide between the two, some varieties responding better to permanganate and the others to manganese dioxide. Manganous sulphate came out next and was followed by manganous carbonate which was slightly better than the control. Among the different varieties, Ponderosa, King Humbert
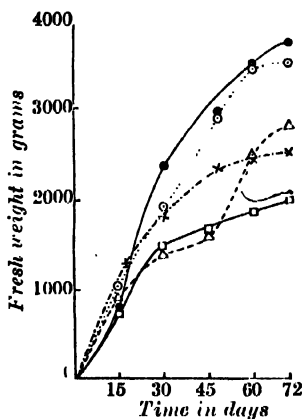
FIG. 11.   Variety—Perfection.
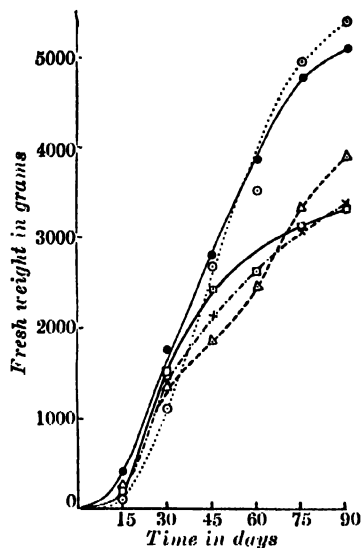
FIG. 12.   Variety—King Humbert.
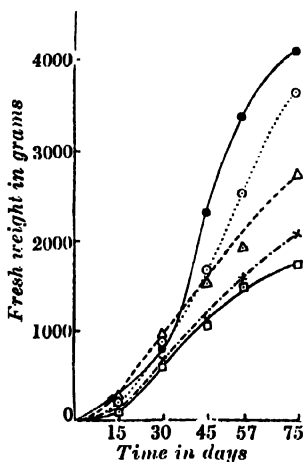
FIG. 13.   Variety—Cherry Red.
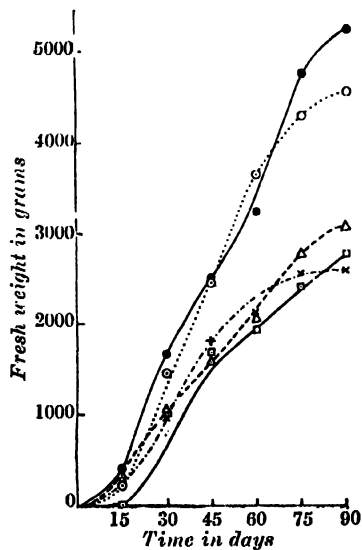
FIG. 14.   Variety—Golden Jubilee.

×—× Control   △--△ MnSO₄   ⊙⊙ KMnO₄   ●—● MnO₂   □—□ MnCO₃

FIGS. 7 to 14.   Effect of different forms of manganese on the yield of tomato.

and Golden Jubilee were the heaviest yielders and responded best to treat-
ment with permanganate or manganese dioxide.
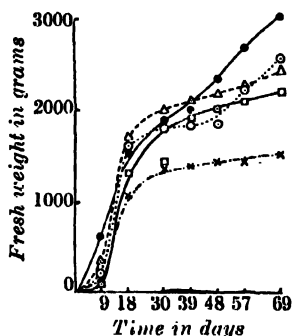
FIG. 15. Variety—Ponderosa.
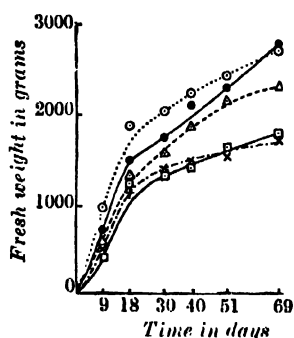
FIG. 16. Variety—Bonnie Best.
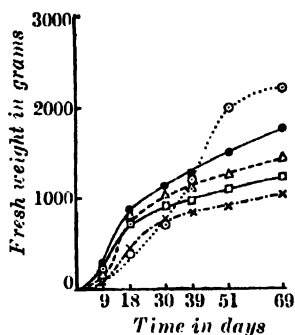
FIG. 17. Variety—Globe.
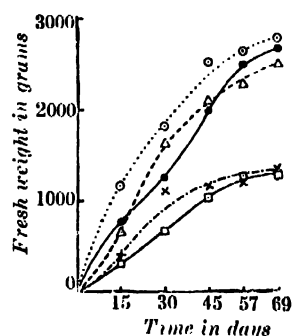
FIG. 18. Variety—Marglobe.

Observations on individual varieties.—

Ponderosa.—In both the limed and the unlimed series, the plants receiving permanganate were the first to bear fruit. They were soon overtaken, however, by those receiving manganese dioxide, which bore very heavily in the course of the first month. After that period there was a short one of rest followed by steady flowering and fruiting almost upto the end. The resting period is noticeable in all the cases, but the effect is least seen in those receiving manganese dioxide. The limed samples followed nearly the same course as the unlimed ones, but the yields were consistently lower than those from the latter.

Bonnie Best.—This variety bore fruit, at a steady rate but the yield, when reckoned on the basis of weight, was rather low. As observed previously, the pots treated with permanganate were the first to bear fruit. Later observations did not however bring out any marked difference between the different treatments. In the unlimed series, permanganate and manganese dioxide were only slightly superior to the other treatments. Liming
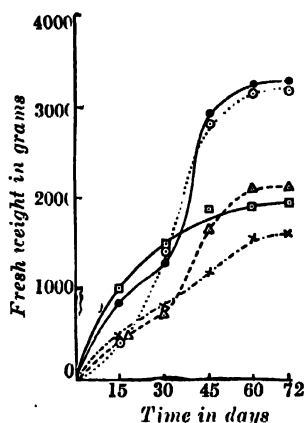
FIG. 19. Variety—Perfection.
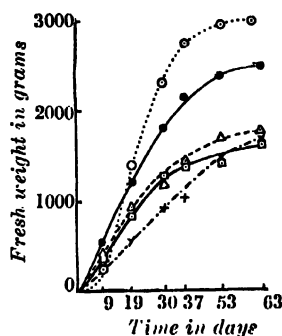
FIG. 20. Variety—King Humbert.
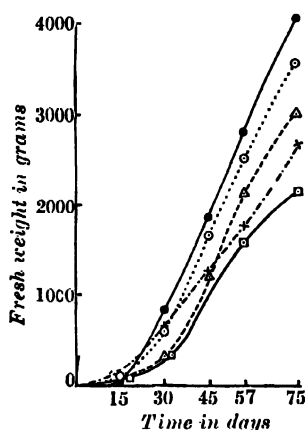
FIG. 21. Variety—Cherry Red.
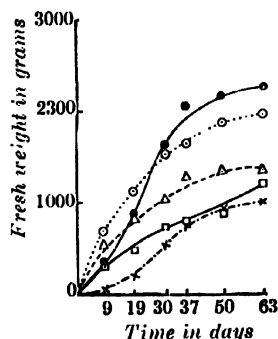
FIG. 22. Variety—Golden Jubilee.

⋯ Control   ▲⋯▲ MnSO₄   ○⋯○ KMnO₄   ●—● MnO₂   ▫—▫ MnCO₃

FIGS. 15 to 22.   Effect of lime on the response of tomato to different forms of manganese.

depressed the yield and the adverse effect was most marked in the case of the controls (untreated) and those receiving manganous carbonate.

Globe and Marglobe.—These two varieties behaved more or less similarly. In the unlimed series, manganese dioxide evoked the best response, though the increase in yield (30–50 per cent.) was not so marked as in the case of some other varieties. In the limed series, the yields were, as usual, greatly depressed. Permanganate yielded the best results and was followed by manganese dioxide and manganous sulphate respectively. Manganous carbonate was not much superior to the control.

Perfection.—In both the limed and unlimed series, manganese dioxide produced the best results, with permanganate following as a close second. The adverse effect of lime was most seen in the case of the controls and those receiving manganous sulphate.

King Humbert.—This variety is a heavy yielder. Application of permanganate increased the yield by about 50 per cent. Manganese dioxide was somewhat less effective but it was nevertheless very much superior to the other treatments. Liming reduced the yield by about 50 per cent., but even there the two oxidisers (permanganate and manganese dioxide) produced the best results.

Cherry Red.—The response of this variety to treatment with different forms of manganese was similar to those of others. Manganese dioxide yielded the best results with permanganate as the second. The effect of lime was however rather unexpected. In three cases, there was distinct increase in yield consequent on that treatment, while in the other two, it remained (permanganate and manganese dioxide) more or less the same. The significance of this observation is rather obscure but it would be of considerable scientific as well as practical interest to compare the chemical composition and physical properties of the tissue fluids of this variety with those of others (especially Golden Jubilee) which are adversely affected by lime.

Golden Jubilee.—This variety was a good, steady yielder and showed practically no lag during the three months of production. It responded best to manganese dioxide. Permanganate was a fairly distant second while the other treatments came very much behind. Liming had the worst effect on this variety, the yields being reduced to less than half by that treatment.

It would thus be seen that all the varieties responded very favourably to treatment with permanganate or manganese dioxide, the yields (as compared with the manured controls) being increased by 30-100 per cent. depending on the nature of the variety. Some varieties responded more favourably to a basal dressing of permanganate than to a top-dressing of manganese dioxide. Since both those treatments are chemically of the same order and yield similar products, it would appear to be advantageous to combine them. The quantities to be thus applied, the influence of season and soil conditions on such treatments would require further study. Except in the case of Cherry Red, liming was consistently unfavourable, but it would nevertheless be useful to determine whether similar observations would apply to other organic manures and to different types of soils.

Unmanured series.—The results of these experiments have not been recorded as the growths were generally poor and the yields low and unsteady. It was observed, however, that both in the limed and the unlimed series, the plants receiving permanganate or manganese dioxide came out better and yielded more fruit than the others.

Effect of treatment with different forms of manganese on the quality of tomato.—The market value of tomato depends largely on the size, shape and colour of the fruit. The popular taste in regard to sweetness and flavour, as also for fleshy or juicy fruits, is highly variable, but it may be stated in general, that sweet and juicy fruits are more favoured than the others. The activity of the digestive ferments present in the fruits, as also their contents of Vitamins B_1 and C are factors determining the nutritive value of fruits.

Specimens of fruits representing different varieties and treatments were compared from time to time. It was observed that shape and colour were essentially varietal characteristics. The size was partly dependent on the manuring. Thus, those receiving cake without lime were generally bigger than those with lime. The latter were, in turn, better than those raised in unmanured pots. No special distinction could be noticed, however, between the different treatments of manganese when the variety and the manuring were the same. The plants receiving permanganate or manganese dioxide bore larger number of fruits than those on other treatments, so this would account for the heavier yields obtained from the former.

The taste and flavour of the fruits representing different treatments were compared in the following manner:--Fruits of nearly the same size and at about the same stage of ripeness were collected at the same time and cut into slices which were kept separately. A number of volunteer tasters, "A", "B", "C", "D",.... were invited to sample the slices and place them in order of taste and flavour. The opinions thus collected were entered separately for each variety and then analysed for their significance. It was found however that the results thus obtained were rather discordant. No special distinction could be found between taste and flavour. The order of preference varied with the individual and there was no clear majority in favour of fruits derived from any treatment. As an instance of the above, the order of taste and flavour as placed by four independent tasters in regard to one variety (King Humbert) may be cited below (Table IX).

It may be seen from the table that the variations, if any, resulting from the different treatments were not so marked as to call for at least a majority opinion. The subtle distinctions observed by the individual tasters should be traced to certain personal factors which are obscure. It has to be

<div align="center">TABLE IX.</div>

Tasters	Treatment				
	Untreated (control)	$MnCO_3$	$MnSO_4$	MnO_2	$KMnO_4$
A	2	4	3	1	5
B	3	5	4	2	1
C	3	4	2	5	1
D	4	2	1	3	2

inferred therefore that the chemicals, by themselves, had no perceptible effect on the taste and flavour of the fruit.

Vitamin contents.—Vitamin B_1 was assayed colorimetrically, the colour index as defined by Ghosh and Dutt (1933) being determined. It was found, however, that the values thus obtained were variable, even parallel samples showing a difference of 20-30 units. Even after allowing for this, some slight difference could be noticed between the controls and the different treatments. Thus, in the case of one variety (Cherry Red), the values were as follows:— Control (untreated), 570 ; $MnCO_3$, 478 ; $MnSO_4$, 504 ; $KMnO_4$, 454 and MnO_2, 463. When different varieties receiving the same treatment ($KMnO_4$) were compared, results of the following type were obtained :—King Humbert, 240 ; Perfection, 230 ; Cherry Red, 450 ; Peach, 440. It may thus be seen that the varietal differences were much greater than those introduced by the treatments.

Similar observations were also made in regard to Vitamin C contents which were titrated according to Birch, Harris and Ray (1933). Each variety had its own small range within which the different values lay. The values represented as mg. of ascorbic acid per c.c. of juice were as follows:— Ponderosa, 0·22-0·23 ; Bonnie Best, 0·22-0·25 ; Globe, 0·26 0·28 ; Marglobe, 0·20-0·21 ; Perfection, 0·20 0·22 ; Cherry Red, 0·25 0·29 ; King Humbert, 0·14-0·17 ; and Golden Jubilee, 0·26 0·28. The following were some typical values for individual treatments.

Variety, Cherry Red.—Control (untreated), 0·28 ; $MnSO_4$, 0·26 ; $KMnO_4$, 0·25 ; MnO_2, 0·28 ; and $MnCO_3$, 0·29.

Golden Jubilee.—Control, 0·27 ; $MnSO_4$, 0·26 ; $KMnO_4$, 0·26 ; MnO_2, 0·26 ; and $MnCO_3$, 0·28.

Bonnie Best.—Control, 0·23; $KMnO_4$, 0·22; MnO_2, 0·25; & $MnCO_3$, 0·22.

Perfection.—Control, 0·20 ; $MnSO_4$, 0·22 ; MnO_2, 0·22 ; and $MnCO_3$, 0·21.

Effect of varying dosages on the yield of French beans and tomato.—In the previous experiments, the different forms of manganese were compared on a more or less arbitrary basis. Thus, permanganate was applied at one-third of a ton per acre and compared with manganous sulphate applied on equivalent manganese basis. That this comparison is not justified is shown by the fact that whereas manganous sulphate continues to remain, at least for a short period, in water soluble form, the permanganate turns almost immediately into manganese dioxide. If manganese in soluble forms has any adverse effect on plant growth, it would naturally be more prominently seen in the case of manganous sulphate than in that of permanganate. It was considered desirable therefore to compare varying dosages of manganous sulphate with another form—manganese dioxide—which was more effective in the previous series. Some pot-culture experiments were accordingly carried out with French beans and tomatoes which had responded favourably in the earlier studies. The details relating to the preparation of the pots were the same as in the previous series except that larger quantities of cake (45 g. per pot) were applied. After applying the usual top-dressings, the pots with the seedlings were divided into six batches of 50 each and treated as follows:—(*a*) MnO_2 at 3·0 g. per pot ; (*b*) MnO_2 at 6·0 g. per pot ; (*c*) MnO_2 at 7·5 g. ; (*d*) $MnSO_4$ at 1·5 g. ; (*e*) $MnSO_4$ at 3·0 g. ; and (*f*) control (untreated). Half the number of pots were allotted to French beans and the other half to tomatoes.

It was observed that the vegetative growth was favoured by increasing quantities of manganese dioxide whereas the reverse was observed in the case of manganous sulphate. The yields were also correspondingly affected as may be seen from Table X.

It may be noted that the yields obtained with 1·5 g. of manganous sulphate were nearly as high as those with 7·5 g. of manganese dioxide.

The foregoing experiments were carried out during the period, May-September, 1934. There were fairly heavy rains during June (18th–30th) when the tomatoes came to flower and the French beans were bearing. It may be expected that the adverse weather conditions had a depressing effect on the yield, but it is hardly probable that it affected any single treatment to a greater extent than the others. It has to be inferred therefore that manganous sulphate in minute quantities may be as beneficial as manganese dioxide or permanganate in much heavier doses.

The conditions relating to the application of soluble forms of manganese and the mechanism of their action on organic matter will form the subjects of later communications.

TABLE X.

Treatment	Total yield in grams	
	Tomato (var.—Golden Jubilee)	French Beans
	(Fresh weight)	(Dry weight)
MnO$_2$ (3 g. per pot).. ..	4995	565
,, (6 g. ,,).. ..	5798	630
,, (7.5 g. ,,).. ..	6113	710
MnSO$_4$ (1.5 g. ,,).. ..	5858	705
,, (3.0 g. ,,).. ..	4125	610
Control (untreated)	3908	530

Discussion.

The present enquiry has brought to light a number of facts of scientific as well as practical importance. It has also indicated certain promising lines of future research.

The plot experiments with ragi have shown that while the yield can be improved by application of either permanganate or manganese dioxide, the extent of benefit to be derived from the treatment is largely determined by the season. This observation is in keeping with the other known facts in fertiliser practice. It is nevertheless important to determine the precise nature of the effect of climatic conditions on the action of oxidisers in the soil. The details relating to the application of the oxidisers in different seasons have yet to be standardised. The nature of the agencies determining the ratio of grain to straw will have to be studied and the conditions modified so as to ensure the best return to the producer.

The mode of interaction between soil and the different compounds of manganese and the subsequent changes in organic matter would suggest that in field practice (*a*) the ionic effect, if any, is much less important than the oxidising action, and (*b*) the resulting insoluble compounds are primarily responsible for the beneficial effects observed. The mechanism of the related changes is still not clear, but some evidence has already been obtained to show that manganese dioxide is one of the products formed in all the cases. Some of the manganese is also present in acid soluble form, but further work is needed to show whether it occurs as compounds such as carbonate and

phosphate or is otherwise associated with the mineral complex of the soil. The present study was carried out with only one type of soil. The observations should be extended to other types, as well, before any definite conclusions can be drawn. The influence of various factors such as reaction, temperature and moisture on the distribution of manganese should be studied. The relation of these changes to the oxidation of organic matter should also be followed.

The observations on the partial sterilising action of some of the compounds of manganese are highly suggestive. It is not clear as to whether the action is selective. The subsequent rapid recovery in numbers and the increased oxidation of organic matter would show that the adverse effect, if any, is only momentary and that the ultimate changes are beneficial to the crop. The action of these compounds is, in some respects, similar to that of those employed to combat soil sickness. It would be of much interest therefore to determine whether the two types of action are identical. It would also be of much practical value to determine whether chemical oxidation can be utilised to combat soil sickness and, better still, to remove the causes which lead to it.

The experiments with tomatoes have conclusively shown that basal dressings of permanganate or top-dressings of manganese dioxide lead to greatly increased yields. Addition of lime has a general depressing effect, but since, even, then the two oxidisers have given the highest yields, it may be expected that their application in field or glass-house practice (as the case may be) will always be attended by beneficial effects. The later observations have shown that small dressings of manganous sulphate can also lead to increased yields, but further work is needed to standardise the conditions for its application.

Tomato is a crop of much economic importance. The fruit is highly nutritious and is consumed in large quantities in all parts of the world. The crop is grown in the open in tropical countries, but in the colder regions it is largely raised under glass. The tomato requires heavy manuring and, at any rate in many parts of Europe and America, is an expensive crop to produce. It may be naturally expected therefore that any treatment which increases the yield by even a small margin should lead to useful returns to the producer. Treatment with oxidisers—especially with minerals such as manganese dioxide or ferric oxide—is comparatively cheap and since the present observations have shown that increased yields ranging from 30 to 100 per cent. (depending on the nature of the variety) can be obtained, such compounds should find ready application both in the field and in glass-house practice.

The beneficial effect of added mineral oxides raises a fresh issue of much scientific as well as practical interest. Every soil contains a useful quantity of ferric iron, at least a part of which is present as the oxide. Many soils are also naturally rich in manganese. These compounds are, no doubt, useful in bringing about a part of oxidation changes as may be seen from the results of the present study. They are not, however, so effective as their total quantities would suggest. They are indeed less useful than the much smaller quantities of fresh oxides applied as top-dressings. It would appear, therefore, that long periods of weathering, as also association with the silicates of the soil, combined with mechanical aggregation, have rendered them comparatively inactive. This, in turn, would naturally suggest that, if by some process, the mineral oxides of the soil can be activated, addition of fresh oxidisers would be largely unnecessary. A number of experiments have accordingly been started, subjecting the soil to a variety of treatments, some of which are similar to those adopted for effecting partial sterilisation. It is hoped that the results of these and other trials will form the subject of a later communication.

In addition to the above, further work is needed to determine the particular form or forms in which a chemical oxidiser would be most effective. Thus, there are several forms of ferric oxide or manganese dioxide all of which may not be equally useful. The method of preparation (if produced in a factory) and the state of division would be important factors determining the efficiency of the oxidiser. When a quarried mineral is used, the associated compounds would also have to be taken into consideration when assessing the fertilising value of the oxidiser.

The previous researches (Bhaskaran, Narasimhamurthy, Subrahmanyan and Sundara Iyengar, 1934 ; Sundara Iyengar and Subrahmanyan, 1935) have also shown that soluble ferrous iron is steadily precipitated in the soil and finally oxidised to the ferric condition. It may be naturally expected that, in such cases, the precipitate will occur in finely divided condition. The resulting oxide may thus prove to be more reactive than any other form which may be directly applied to the soil. Some experiments have therefore been started, comparing the oxidative efficiency of different forms of soluble ferrous as well as ferric iron with that of pure ferric oxide. Similar trials are also being conducted with different soluble manganese salts comparing them with manganese dioxide.

Although there has been no significant difference in regard to Vitamin B_1 and C contents, it is yet probable that application of different forms of manganese may have produced other profound changes in plant metabolism and modified the nutritive value of the products. The effect on enzyme

activity is still awaiting systematic investigation. Some preliminary observations suggested that there is no appreciable difference in regard to oxidase and peroxidase activities, but further quantitative work is needed before any definite conclusion can be reached. The distribution of manganese between the different parts of the plant will also be of much value, in view of the increasing importance of manganese in animal and human nutrition.

Summary.

(1) Plot experiments with ragi (*Eleucine coracana*) showed that, on both manured and unmanured soil, treatment with small quantities of permanganate led to increased yields of grain and straw. Permanganate applied together with the organic manure was more effective than that applied later as top-dressing.

(2) Permanganate applied to soil passes rapidly into water-insoluble condition. Part of the product is soluble in dilute acid, while the rest is insoluble in that reagent. Other compounds of manganese also behave in a manner similar to that of permanganate. Manganous sulphate is slow to react, but after a few days, that too yields insoluble products.

(3) Application of either potassium permanganate or manganous sulphate causes an immediate reduction in the number of soil bacteria. After a few days, however, the adverse effect is removed and the numbers increase at a rapid rate.

(4) When equivalent quantities of different forms of manganese were applied, the decomposition proceeded most rapidly in presence of manganese dioxide. Permanganate came second and was followed by manganous carbonate and manganous sulphate respectively.

(5) Production of carbon dioxide followed the same order as the decomposition of organic matter. There was close correlation between loss of carbon on the one hand and production of carbon dioxide on the other.

(6) Under the conditions of the present study, less than a fourth of the oxidation of organic matter was due to the action of the mineral constituents of the soil. About a third was due to the oxidiser when manganese dioxide was applied. The rest was due to microbial action. The division is however only arbitrary since the various agencies are either mutually dependent upon or otherwise influenced by each other.

(7) Experiments with eight varieties of tomato have convincingly demonstrated the advantages of supplementing organic manures with chemical oxidisers. Improved yields ranging from 30 to 100 per cent. (depending on the variety) were obtained. Manganese dioxide and potassium permanganate yielded the best results. Manganous carbonate and manganous

sulphate—at any rate, in the proportions at which they were tried—were not much superior to the control (untreated). Among the different varieties, the best response was from Ponderosa, King Humbert and Golden Jubilee.

(8) Liming generally depressed the yield of tomato. The adverse effect was greatly reduced by application of either manganese dioxide or potassium permanganate. The variety, Cherry Red, was, however, an exception as it responded favourably to application of lime.

(9) Treatment with different forms of manganese does not produce any appreciable difference in the quality of tomato. Fruits of the same variety possess about the same degree of flavour and taste and contain approximately the same amounts of Vitamins B_1 and C irrespective of the form of manganese received by them.

(10) The significance of the foregoing and other observations has been discussed. The possibility of applying chemical oxidisers to obtain greatly increased yields of crop—especially of tomato— has been indicated. Certain useful lines of future research leading to (*a*) improved methods of applying chemical oxidisers and (*b*) enhancement of the oxidising action of minerals already present in the soil, have been suggested.

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PROCEEDINGS

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CONTENTS

RAMAN SPECTRA OF ISOPRENE, DIPENTENE AND OCIMENE.

BY P. S. SRINIVASAN.

(From the Department of Physics, Indian Institute of Science, Bangalore.)

1. Experimental.

THE present investigation was undertaken as part of a scheme for the investigation of the nature and physical properties of rubber. As is well known, rubber is a high-molecular weight polymer of isoprene and as a preliminary it was thought worthwhile to study the Raman effect in isoprene (C_5H_8), dipentene ($C_{10}H_{16}$) and ocimene ($C_{10}H_{16}$) of which isoprene alone has already been studied by Dadieu and Kohlrausch[1] while the other two which are its isomeric polymers have been studied now for the first time.

All the compounds being liquids, the well known method of obtaining the Raman spectrum by focussing the image of a mercury arc into the liquid contained in a Wood's tube by means of a condensing lens and then focussing the transversely scattered light on to the slit of a spectrograph, was used. The liquids were all distilled in vacuo a number of times to render them dust-free.

A Hilger two-prism spectrograph was used in the case of isoprene while a slightly higher dispersion Feuss spectrograph was used in the case of the other two liquids. Throughout hypersensitive panchromatic plates were employed. For determining the wave-length a comparison iron arc was taken on each plate and the lines were measured by interpolation.

Of the substances employed, isoprene was prepared chemically pure by the author according to the method described by Basset and Williams.[2] Pale crepe rubber cut into pieces of about 1 gm. each was dropped into a specially constructed iron retort maintained electrically at 600°C., one piece at a time and the products of cracking were collected in two systems of condensers, one maintained at 50°C. and the other in ice and salt. The product collected in the cold condensers was fractionated a number of times and the fraction boiling steadily at 32°·5 C. (680 mm.) was collected.

[1] Dadieu and Kohlrausch, *Ber. Deut. Chem. Gess.*, 1930, **63** (3), 1657.

[2] Basset and Williams, *J. C. S.*, 1932, p. 2324.

The dipentene used in the experiment was a sample obtained as pure from E. de Haen and it was further purified by two distillations under reduced pressure over sodium.

The ocimene was prepared in a very pure state and lent to the author for this work by Mr. B. Sanjiva Rao of the Organic Chemistry Department of the Institute and the author wishes to take this opportunity of thanking Mr. Sanjiva Rao for his kindness.

2. Results.

In the following tables the various exciting lines of the mercury arc are designated by the letters of the alphabet; thus a, b, c, d, e, f, g, h, j, k and l stand respectively for 3650·3, 3654·9, 3663·3, 3984·1, 4046·8, 4078·1, 4339·5, 4347·7, 4358·6, 4916·4 and 5461.

The numerical subscripts attached to the several letters indicate the relative intensities of the Raman lines excited by the corresponding mercury line on an arbitrary scale wherein the intensity of the Rayleigh scattered 4916 line is taken as 10.

Table I gives the Raman frequencies of isoprene.

TABLE I.

$CH_2 = C - CH = CH_2$ *Isoprene.*
$|$
CH_3
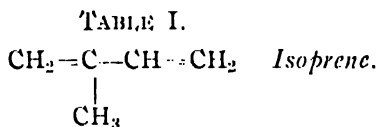
No.	Shift ν cm.$^{-1}$	Exciting lines with intensity	No.	Shift ν cm.$^{-1}$	Exciting lines with intensity
1	525·4	$c_1 j_1$	8	1382·3	h_1 (?)
2	778	j_2	9	1422·4	$e_3 j_3$
3	899·8	$e_2 j_2$	10	1636·8	j_{10}
4	950·8	$c_1 j_1$	11	2911·3	e_3
5	991·1	$e_1 j_1$	12	2981·2	b_2
6	1066·6	$e_2 j_1$	13	3015·7	$e_5 j_4$
7	1292·5	$e_5 j_5$	14	3092	e_2

Table II gives a comparison of the frequency shifts obtained by the author with those obtained by Dadieu and Kohlrausch, the figures in the brackets indicating the intensities of the respective Raman lines. Of the two low frequencies 289 and 423 reported by Dadieu and Kohlrausch and

not appearing in the present work, the former is considered uncertain even by Dadieu and Kohlrausch themselves. The agreement otherwise is good.

<div align="center">TABLE II.</div>

<div align="center">*Comparison Table for Isoprene.*</div>

No.	Author	Dadieu and Kohlrausch	No.	Author	Dadieu and Kohlrausch
1	....	289 (1) (?)	9	1292·5 (5)	1291 (5)
2	....	423 (2)	10	1382·3 (1)	1380 (1)
3	525.1 (4)	530 (4)	11	1422·4 (3)	1420 (5)
4	778 (2)	779 (2)	12	1636·8 (10)	1636 (10)
5	899·8 (2)	899 (3)	13	2914·3 (3)	2920 (2)
6	950·8 (1)	954 (4)	14	2981·2 (2)	2983 (1)
7	991·1 (1)	991 (2)	15	3015·7 (5)	3010 (5)
8	1066·6 (1)	1070 (4)	16	3092 (2)	3083 (3)

Table III gives the results for dipentene using the same notation as before.

<div align="center">TABLE III.</div>

$$CH_3 - C \overset{CH-CH_2}{\underset{CH_2-CH_2}{\diagdown}} CH-C \overset{CH_2}{\underset{CH_3}{\diagup}} \quad Dipentene.$$

No.	Shift ν cm.$^{-1}$	Exciting lines with intensities	No.	Shift ν cm.$^{-1}$	Exciting lines with intensities
1	635·7	$c_1 j_{0.5}$	9	1306·9	$c_1 j_2$
2	673·7	$c_1 j_{0.5}$	10	1375	$c_1 j_2$
3	713·7	$c_1 j_{0.5}$	11	1440·2	$c_1 j_2$
4	760·1	$c_2 j_3$	12	1610·6	$e_3 j_6 g_1 h_1$
5	818·5	$c_1 j_{0.5}$	13	1673 (broad)	j_1
6	879·2	$c_2 j_3$	14	2868·5	$e_3 j_2$
7	1051·5 (band)	$j_{0.5}$	15	2922	$e_3 j_2$
8	1207·3	$c_1 j_3$	16	2971·5	$e_3 j_2$

The various frequency shifts obtained with ocimene are given in Table IV.
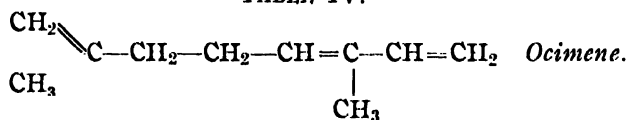
TABLE IV.

$$CH_2 \diagdown$$
$$CH_3 \diagup C—CH_2—CH_2—CH=C—CH=CH_2 \quad \textit{Ocimene.}$$
$$\underset{CH_3}{|}$$

No.	Shift ν cm.$^{-1}$	Exciting lines with intensities	No.	Shift ν cm.$^{-1}$	Exciting lines with intensities
1	433	$e_1 j_1$	9	1376·6	$e_1 j_1$
2	752·8	$e_{0.5} j_{0.5}$	10	1406·1	$e_3 j_2$
3	885·1	$e_1 j_1 g_{0.5} h_{0.5}$	11	1440	$j_1 d_{0.5}$
4	959·1	$e_1 j_2$	12	1634	$e_4 j_7 g_5 h_1$
5	1069·5	$e_1 j_1$	13	1666	j_3
6	1235·3	$e_2 j_3$	14	2912	$e_3 j_1$
7	1285·3 (band)	$e_2 j_1$	15	2983	j_1
8	1314·4	$e_1 j_{0.5}$	16	3055	$e_1 j_1$

3. Discussion.

(a) Relation to Chemical Constitution.

Isoprene is represented by the formula C_5H_8 and it has therefore 33 degrees of freedom which should all be represented in the Raman spectrum. Some of these might be forbidden and some others might be duplicated so that all the 33 frequencies cannot be reasonably expected. It is however remarkable that only 14 frequencies have been observed by the author while Dadieu and Kohlrausch have observed only one more.

It is well known that the frequency of oscillation of two atoms or groups of atoms can be calculated from a knowledge of the binding force between them and their masses by means of the formula

$$\nu = \frac{1}{2\pi}\sqrt{\frac{f}{\mu}}$$

or from the heat of dissociation of the respective groups from the formula

$$\nu = \kappa\sqrt{\frac{\Lambda}{\mu}}$$

where ν represents the frequency of oscillation ;
f represents the binding force ;

μ represents the reduced mass ;

A represents the energy of dissociation ;

and K is a constant equal to 291 ·5.

A large number of calculations has been made for various bonds and groups by different authors, notably by Dadieu and Kohlrausch[3] and fair agreement with observations has been obtained. In the following table (Table V) are given different linkages with their characteristic oscillations, both calculated by the two formulæ and observed. The groups compared are C—H ; C—C ; C=C ; —C—(transverse) ; H—CH—CX₂ and the structure $>C=C-C<$.

TABLE V.
Frequencies and Linkages.

No.	Type of Linkage	Δν deduced from			Δν observed		
		Binding Force	Thermal Dissociation	Group Effect	Isoprene	Dipentene	Ocimene
1	C—H (aliphatic)	2920	2988	..	2914 2981 3015	2685 2922 2971	2912 2983
2	C—H (aromatic)	3050	3118	..	..	..	..
3	H —C—(transverse) H	..	..	1200-1500	1382 1422	1207 1306 1375 1440	1235 1344 1376 1406 1440
4	H—CH=CX₂	..	..	3080	3092	..	3055
5	C—C (aliphatic)	890	900	..	899 ·8	879·2	885 ·4
6	C=C	1620	1635	..	1636	1610	1634
7	>C=C—C<	..	..	760 1070 1290	778 1066 1292	..	752 1069 1285

It is evident from the above table that the three compounds examined give the characteristic frequencies for C—H (aliphatic), C—C (aliphatic) and

[3] Kohlrausch, *Der Smekal-Raman Effect.*

C — C unmistakably in the correct regions and the transverse C— H oscillations have also come out in the three cases. The table further makes it evident that the shifts 3092 and 3055 observed in isoprene and ocimene which are too high for aliphatic C—H oscillations are really due to the presence in those compounds of the group $II \cdot CH : CX_2$. The group of frequencies 760,

1070 and 1290 characteristic of the structure $> C — \overset{|}{C} — C <$ comes out in the

case of isoprene and ocimene but not in dipentene thus lending support to the chemical structure assigned to these compounds.

It is worthy of remark that in the case of ocimene there are two shifts in the C — C region, one having a value 1634 and the other 1666. There are in the ocimene molecule three double bonds of which two are conjugate as in isoprene while the third is simple. The conjugate double bonds give rise to the 1634 oscillation while the other one gives an exalted frequency probably due to the loading of the bond with the CH_3 and CH_2 radicles.[4] The intensity of the 1634 oscillation is nearly twice that of the 1666 oscillation thus lending further support to the consideration that the former is due to the two conjugated double bonds while the latter is due to the simple bond.

It is interesting to note that dipentene, a compound known to possess a cyclic structure does not give the characteristic frequencies given by benzene or toluene. Like cyclo-hexane, dipentene has nothing in common with benzene except that there are six carbon atoms joined up in a ring. It is also well known that the properties of dipentene well accord with those of aliphatic compounds. Quite probably the ring structure in dipentene unlike that in benzene is a puckered one similar to that in cyclo-hexane with the carbon atoms not all in one plane. The non-benzenoid character of the ring in dipentene is independently borne out also by X-ray diffraction results.[5]

(b)   Relation to Infra-Red Absorption.

It is well known that the Raman spectrum gives only the fundamental oscillations of a molecule, the harmonics and the combinations occurring very rarely or never at all. Such being the case, it would be of interest to compare the Raman spectra with the infra-red absorption spectra.

Unfortunately infra-red absorption data are not available at all for isoprene and ocimene while for dipentene the results of Coblentz[6] on d-limonene have been taken as not far different. The chemical structure of dipentene

4 Daure, Introduction a l'etude de l'effect Raman, p. 70.

5 V. I. Vaidyanathan, Indian Journal of Physics, 1929, 3, 387.

6 Coblentz, Investigations of Infra-red Spectra, 1905, p. 92.

and limonene is identical and further Hantzsch[7] has observed that with increasing purity the absorption of dipentene comes to agree with that of limonene. Table VI contains the infra-red peaks converted into frequencies along with the Raman lines given by dipentene. Coblentz has investigated this compound up to 15μ and hence it is impossible to answer satisfactorily for all low frequencies, lower than about 750. The letters in brackets indicate the strength of the absorption maxima while the figures in brackets indicate the intensity of the Raman lines.

TABLE VI.

Raman Frequencies and Infra-Red Absorption Frequencies—Dipentene.

No.	Infra-red Absorption		Raman Frequency ν cm.$^{-1}$	No.	Infra-red Absorption		Raman Frequency ν cm.$^{-1}$
	μ	Frequency ν cm.$^{-1}$			μ	Frequency ν cm.$^{-1}$	
1	..	..	636 (0.5)	11	8·35	1198 (*w.*)	1207 (3)
2	..	..	674 (0.5)	12	7·6	1315 (*w.*)	1307 (2)
3	..	..	714 (0.5)	13	7·2	1389 (*m.*)	1375 (2)
4	12·7	787 (*s.*)	760 (3)	14	6·95	1438 (*w.*)	1440 (2)
5	..	..	818 (0.5)	15	6·2	1610 (*m.*)	1610 (6)
6	11·3	885 (*v.s.*)	879 (3)	16	6·05	1652 (*m.*)	1673 (1)
7	10·5	952 (*w.*)	..	17	5·2	1923 (*v.w.*)	..
8	9·7	1030 (*m.*)	1051 (0.5)	18	3·9	2563 (*w.*)	..
9	8·9	1123 (*m.*)	..	19	3·45	2898 (*s.*)	2869 (2)
10	8·7	1149 (*w.*)	..				2922 (2)
							2972 (2)

(Letters in the third and seventh columns signify the following: —*v.s.*, very strong; *s.*, strong; *m.*, medium; *w.*, weak; *v.w.*, very weak.)

It is well known that the symmetric oscillations involving no change in the electric moment of the molecule come out in great strength in Raman spectra while they are entirely absent in the infra-red absorption spectra, *e.g.*, 992 of benzene. It is also well known that certain frequencies occurring in the infra-red but not in scattering can be explained as combinations of some fundamentals.

[7] Hantzsch, *Ber. Deuts. Chem. Gess.*, 1912, **45** (1), 553.

Any oscillation of dipentene, a highly unsymmetrical molecule, will produce large changes in the electric moment of the molecule and as such we should expect that all the Raman frequencies will be fairly well represented in the infra-red absorption. The table shows good general agreement both in number and shifts.

The maximum 2563 in the infra-red has no analogue in the Raman spectrum. This can be interpreted as a combination of 879 and 1673 ($=2552$), two oscillations of the molecule, one of which is the C—C. The other neighbouring infra-red maximum at 1923 can also be interpreted as a combination of the C—C oscillation 879 with a minor oscillation 1051 making the combination tone equal to 1930. The absence in the Raman spectrum of the infra-red maxima at 1149 and 952 may be explained by the fact that they are quite weak in the absorption itself and might come out probably after very long exposure in scattering. It is rather difficult to understand why the infra-red frequency at 1123 which is of moderate intensity in absorption does not come out in the Raman spectrum, especially as no combinations with low frequencies usually occur. The Raman frequency 818 does not come out in the infra-red probably because it is a very weak oscillation even in scattering and perhaps a very careful investigation in this region of absorption might show a weak maximum. Evidently it cannot be a symmetric oscillation, being very weak in scattering itself.

The general agreement, however, is quite satisfactory.

Fig. 1. Isoprene
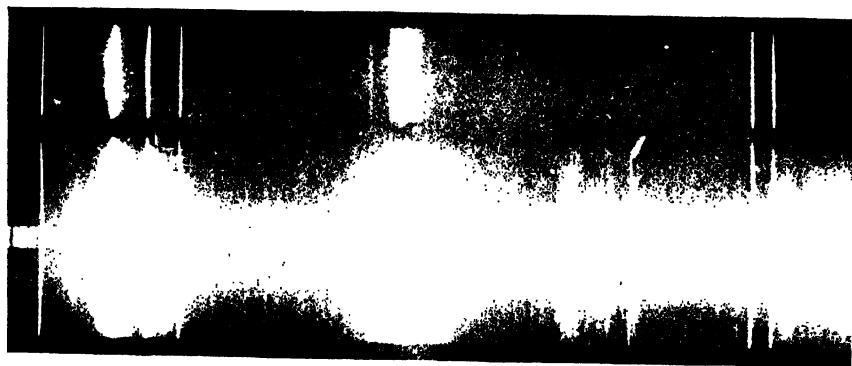
Fig. 2. Dipentene.

Fig. 3. Ocimene.

RAMAN SPECTRA.

ANALYSIS OF THE SPECTRUM OF TREBLY IONISED ZINC : Zn IV.

By T. S. SUBBARAYA, M.Sc., A.Inst.P.,

Central College, University of Mysore, Bangalore.

THE spectrum of trebly ionised Zinc should be similar to that of trebly ionised Mercury and Cadmium. An analysis of the Hg IV spectrum was reported some time ago[1] ; the present paper is concerned with an analysis of Zn IV. Cd IV has also been analysed and will be published shortly.

The wave-length data have been taken from R. O. Hutchinson.[2] These measurements are not accurate and the wave-lengths differ sometimes from those of Laporte and Lang[3] by more than 0·5 Å in the region common to the two. A similar error is even more serious in the case of Zn IV lines which are of shorter wave-length. Accordingly the common differences sometimes vary by as much as 40 cm.⁻¹ so that mere recurring differences are not to be relied on for purposes of classification. A consideration of the intensity and completeness of the multiplets is a surer indication of the correctness of the analysis. Another criterion is furnished by the question whether almost all the lines in the appropriate region are covered by the scheme. Thus the extreme ultraviolet spectra may be divided up into consecutive regions which belong almost exclusively to one stage in a series of successive stages of ionisation. A correct analysis embraces almost all the lines in one of these regions without leaving serious gaps. This is no doubt due to the fact that in any stage of ionisation only the low and middle levels are excited so that stray lines due to high levels not included in the classification and obtruding amongst the classified lines are quite rare. Though some apparently correct multiplets may be found by chance on account of the unreliability of the constant differences, all the important lines will not conform to the classification unless the intervals have been correctly chosen. Thus the region from λ 1768 to λ 1457 is almost wholly made up of Zn III lines and the correctness of the analysis is shown by the fact that the low and middle terms account for nearly all these lines. Similarly the spectrum of Hg IV was found to embrace nearly all the lines in the region λ 1658 to λ 1100 as combinations between low and

[1] *Proc. Ind. Acad. Sci.*, 1934, 1, 39.

[2] *Astrophys. Jour.*, 1923, 58, 280.

[3] *Phys. Rev.*, 1927, 30, 328.

TABLE I.

	d's				d⁷s²			
	$^4F_{9/2}$	$^4F_{7/2}$	$^4F_{5/2}$	$^4F_{3/2}$	$^4F_{9/2}$	$^4F_{7/2}$	$^4F_{5/2}$	$^4F_{3/2}$
$^4F^\circ_{9/2}$	(4) 81719 *5935* *8041*	..	..	..	(4) 79170 *5057* *3053*	(1) 74113 *3065*	..	..
$^4F^\circ_{7/2}$	(1) 84760 *5945*	(3) 78815 *3295* *1817*	(0) 75517 *1835*	..	(1) 82223 *5045*	(3) 77178 *1505*	?	..
$^4F^\circ_{5/2}$	..	(0) 80632 *3350*	(3) 77332 *2775* *1178*	(1) 74627 *1171*	..	(3) 78083 *2319*	(1) 76664 *1032* *1187*	(2) 75632 *1197*
$^4F^\circ_{3/2}$	..	..	(1) 78530 *2732*	(2) 75798	..	..	(1) 77851 *1022*	(1) 76829
$^4G^\circ_{11/2}$	(4) 79751 *3534*	..	..	..				
$^4G^\circ_{9/2}$	(2) 83285 *5933* *4013*	(3) 77352* *4015*	..	..				
$^4G^\circ_{7/2}$	(2) 87298 *5931*	(3) 81367* *3291* *3667*	(1) 78076 *3653*	..				
$^4G^\circ_{5/2}$	..	(3) 85034 *3315*	(4) 81719* *2736*	(3) 78983*				
$^4D^\circ_{7/2}$	(1) 82447 *5042*	(2) 76505 *3299* *3463*	(1) 73206 *3458*	..				
$^4D^\circ_{5/2}$	..	(1) 79968 *3304*	(1) 76664* *2754* *1412*	(1) 73910 *1431*				
$^4D^\circ_{3/2}$		..	(1) 78076* *2735*	(0) 75341 *1323*				
$^4D^\circ_{1/2}$			..	(1) 76664**				

Row groups (left margin): d_8p (for the $^4F^\circ$ block), d_8p (for the $^4G^\circ$ block), d_8p (for the $^4D^\circ$ block).

* Blend.

middle terms. After a few trials an arrangement of multiplets was found in Zn IV which had this satisfactory property of including almost all the lines in the region from λ 1443 to λ 1030. The multiplets are given in Table I. Some combinations are represented by blends with others; this is due to insufficient resolution as can be easily seen by the fact that four pairs of close lines resolved by Laporte and Lang have been noted as single by Hutchinson. The terms deduced from these multiplets are given in Table II. By extend-

TABLE II. *Term Values in Zn IV.*

Configuration	Term	Value	Configuration	Term	Value
$3d^8 4s$	$^4F_{9/2}$	0	$3d^8 4p$	$^4G^o_{11/2}$	79751
	$^4F_{7/2}$	5938		$^4G^o_{9/2}$	83291
	$^4F_{5/2}$	9230		$^4G^o_{7/2}$	87299
	$^4F_{3/2}$	11962		$^4G^o_{5/2}$	90959
$3d^7 4s^2$	$^4F_{9/2}$	2539	$3d^8 4p$	$^4F^o_{9/2}$	81713
	$^4F_{7/2}$	7596		$^4F^o_{7/2}$	84757
	$^4F_{5/2}$	9915		$^4F^o_{5/2}$	86579
	$^4F_{3/2}$	10942		$^4F^o_{3/2}$	87759
$3d^8 4s$	$^2F_{7/2}$	11240	$3d^8 4p$	$^4D^o_{7/2}$	82443
	$^2F_{5/2}$	13840		$^4D^o_{5/2}$	85898
$3d^8 4s$	$^4P_{5/2}$	12550		$^4D^o_{3/2}$	87311
	$^4P_{3/2}$	16610		$^4D^o_{1/2}$	88603
	$^4P_{1/2}$	17630	$3d^8 4p$	$^2F^o_{7/2}$	94858
$3d^9$?	$^2D_{5/2}$?	−10512		$^2F^o_{5/2}$	99267

ing the classification to include the lines given by Bloch and Bloch[4] in the visible and near ultraviolet it is hoped shortly to improve the accuracy of these terms. Since no Rydberg sequences have been found and so no absolute term values are known, there is no point in drawing a $\sqrt{\frac{T}{R}}$ diagram. The course of the $d^8s^4F_{9/2} - d^8p^4F_{9/2}^o$ &c. lines and the various intervals in the iso-electronic-sequence Co I[5], Ni II[5], Cu III[6] and Zn IV are, however, given in Tables III and IV. Table V gives all the classified lines with their assignments.

[4] *Jour. de Physique*, 1934, **5**, 289.
[5] Goudsmit and Bacher, *Atomic Energy States*, McGraw Hill Co.
[6] B. V. Raghavendra Rao, *Zs. f. Phys.*, 1934, **88**, 135.

TABLE III.

Combination	Co I	Diff.	Ni II	Diff.	Cu III	Diff.	Zn IV
d⁸s⁴F₉/₂−d⁸p⁴F°₉/₂ ..	29359	10804	46163	18384	64547	17166	81713
d⁸s⁴F₉/₂−d⁸p⁴G°₉/₂ ..	28982	15989	44971	..	..	..	83291
d⁸s⁴F₇/₂−d⁸p⁴D°₇/₂ ..	..	..	42228	..	..	..	76505

TABLE IV.

Term	Co I	Δν	Ni II	Δν	Cu III	Δν	Zn IV	Δν
d⁸s ⁴F₉/₂	3482·76		8392·9		0		0	
		·659·85		−936·4		−1745		−5938
⁴F₇/₂	4142·61		9329·3		1745		5938	
		547·49		−785·4		−1330		·3292
⁴F₅/₂	4690·10		10114·7		3075		9230	
		−385·65		584·3		−855		−2732
⁴F₃/₂	5075·75		10663·0		3930		11962	
d⁸s ²F₇/₂	7442·39		13549·1		7065		11240	
		−1018·38		−1445·3		−1745		··2600
²F₅/₂	8460·77		14994·4		8810		13840	
d⁸s ⁴P₅/₂	13795·44		25034·6		..		12550	
		−240·76						−4060
⁴P₃/₂	14036·20		24786·9		..		16610	
		362·95						−1020
⁴P₁/₂	14399·15		24834·7		..		17630	
d⁸p ⁴G°₁₁/₂	32430·56		53495·6		..		79751	
		−34·10						3540
⁴G°₉/₂	32464·66		53364·0		..		83291	
		−708·64						−4008
⁴G°₇/₂	33173·30		54261·5		..		87299	
		−501·02						− 3660
⁴G°₅/₂	33674·32		55017·6		..		90959	
d⁸p ⁴F°₉/₂	32841·91		54556·1		64547		81713	
		−315·17		−860·6		−1480		−3044
⁴F°₇/₂	33466·78		55416·7		66027		84757	
		−479·03		−657·3		−758		−1822
⁴F°₅/₂	33945·81		56074·0		66785		86579	
		·250·30		349·4		−462		−1180
⁴F°₃/₂	34196·11		56423·4		67247		87759	
d⁸p ⁴D°₇/₂	..		51556·9		..		82443	
				−1180·5				−3455
⁴D°₅/₂	..		52737·4		..		85898	
				−895·5				··1413
⁴D°₃/₂	..		53633·9		..		87311	
				−541·0				−1292
⁴D°₁/₂	..		54174·9		..		88603	
d⁸p ²F°₇/₂	35450·51		57079·1		66342		94858	
		−879·28		−1412·7		−2023		−4409
²F°₅/₂	36329·79		58491·8		68365		99267	

TABLE V.

List of Classified Lines of Zn IV.

Int.	Wave-length	Wave-number	Classification
2	1443·0	69300	d^8s $^4P_{3/2}-d^8p$ $^4D^\circ_{5/2}$
0	1434·8	69696	d^8s $^4P_{1/2}-d^8p$ $^4D^\circ_{3/2}$
1	1430·7	69896	d^8s $^4P_{5/2}-d^8p$ $^4D^\circ_{7/2}$
0	1414·4	70701	d^8s $^4P_{3/2}-d^8p$ $^4D^\circ_{3/2}$
1	1409·1	70967	d^8s $^4P_{1/2}-d^8p$ $^4D^\circ_{1/2}$
1	1388·8	72005	d^8s $^4P_{3/2}-d^8p$ $^4D^\circ_{1/2}$
3	1387·8	72057	$\begin{cases} d^8s & ^2F_{5/2}-d^8p & ^4D^\circ_{5/2} \\ d^8s & ^2F_{7/2}-d^8p & ^4G^\circ_{9/2} \end{cases}$
2	1374·8	72738	d^8s $^2F_{5/2}-d^8p$ $^4F^\circ_{5/2}$
1	1366·0	73206	d^8s $^4F_{5/2}-d^8p$ $^4D^\circ_{7/2}$
1	1362·9	73373	d^8s $^4P_{5/2}-d^8p$ $^4D^\circ_{5/2}$
2	1360·2	73519	d^8s $^2F_{7/2}-d^8p$ $^4F^\circ_{7/2}$
1	1353·0	73910	d^8s $^2F_{5/2}-d^8p$ $^4F^\circ_{3/2}$
1	1349·3	74113	d^7s^2 $^4F_{7/2}-d^8p$ $^4F^\circ_{9/2}$
1	1340·0	74627	d^8s $^4F_{3/2}-d^8p$ $^4F^\circ_{5/2}$
0	1327·3	75341	d^8s $^4F_{3/2}-d^8p$ $^4D^\circ_{3/2}$
0	1324·2	75517	d^8s $^4F_{5/2}-d^8p$ $^4F^\circ_{7/2}$
2	1322·2	75632	d^7s^2 $^4F_{3/2}-d^8p$ $^4F^\circ_{5/2}$
1	1319·6	75781	d^8s $^4F_{7/2}-d^8p$ $^4F^\circ_{9/2}$
2	1319·3	75798	d^8s $^4F_{3/2}-d^8p$ $^4F^\circ_{3/2}$
2	1307·1	76505	d^8s $^4F_{7/2}-d^8p$ $^4D^\circ_{7/2}$
1	1304·4	76664	$\begin{cases} d^8s & ^4F_{5/2} & d^8p & ^4D^\circ_{5/2} \\ d^7s^2 & ^4F_{5/2}-d^8p & ^4F^\circ_{5/2} \end{cases}$
1	1301·6	76829	d^7s^2 $^4F_{3/2}-d^8p$ $^4F^\circ_{3/2}$
3	1295·7	77178	d^7s^2 $^4F_{7/2}-d^8p$ $^4F^\circ_{7/2}$
3	1292·8	77352	$\begin{cases} d^8s & ^4F_{7/2}-d^8p & ^4G^\circ_{9/2} \\ d^8s & ^4F_{5/2}-d^8p & ^4F^\circ_{5/2} \end{cases}$
1	1284·5	77851	d^7s^2 $^4F_{5/2}-d^8p$ $^4F^\circ_{3/2}$
1	1280·8	78076	$\begin{cases} d^8s & ^4F_{5/2}-d^8p & ^4G^\circ_{7/2} \\ d^8s & ^4F_{5/2}-d^8p & ^4D^\circ_{3/2} \end{cases}$

TABLE V (*contd.*)

Int.	Wave-length	Wave-number	Classification
1	1273·4	78530	$d^8s\ {}^4F_{5/2} - d^8p\ {}^4F^o_{3/2}$
3	1268·8	78815	$d^8s\ {}^4F_{7/2} - d^8p\ {}^4F^o_{7/2}$
3	1266·1	78983	$\left\{ \begin{array}{l} d^8s\ {}^4F_{3/2} - d^8p\ {}^4G^o_{5/2} \\ d^7s^2\ {}^4F_{7/2} - d^8p\ {}^4F^o_{5/2} \end{array} \right\}$
4	1263·1	79170	$d^7s^2\ {}^4F_{9/2} - d^8p\ {}^4F^o_{9/2}$
4	1253·9	79751	$d^8s\ {}^4F_{9/2} - d^8p\ {}^4G^o_{11/2}$
1	1250·5	79968	$d^8s\ {}^4F_{7/2} - d^8p\ {}^4D^o_{5/2}$
0	1240·2	80632	$d^8s\ {}^4F_{7/2} - d^8p\ {}^4F^o_{5/2}$
0	1234·4	81011	$d^8s\ {}^2F_{5/2} - d^8p\ {}^2F^o_{7/2}$
3	1229·0	81367	$d^8s\ {}^4F_{7/2}\ d^8p\ {}^4G^o_{7/2}$
4	1223·7	81719	$\left\{ \begin{array}{l} d^8s\ {}^4F_{9/2} - d^8p\ {}^4F^o_{9/2} \\ d^8s\ {}^4F_{5/2}\ d^8p\ {}^4G^o_{5/2} \end{array} \right\}$
1	1216·2	82223	$d^7s^2\ {}^4F_{9/2} - d^8p\ {}^4F^o_{7/2}$
1	1212·9	82447	$d^8s\ {}^4F_{9/2} - d^8p\ {}^4D^o_{7/2}$
2	1200·7	83285	$d^8s\ {}^4F_{9/2} - d^8p\ {}^4G^o_{9/2}$
2	1195·8	83626	$d^8s\ {}^2F_{7/2}\ d^8p\ {}^2F^o_{7/2}$
1	1179·8	84760	$d^8s\ {}^4F_{9/2} - d^8p\ {}^4F^o_{7/2}$
3	1176·0	85034	$d^8s\ {}^4F_{7/2} - d^8p\ {}^4G^o_{5/2}$
1	1170·6	85426	$d^8s\ {}^2F_{5/2} - d^8p\ {}^2F^o_{5/2}$
2	1145·5	87298	$d^8s\ {}^4F_{9/2} - d^8p\ {}^4G^o_{7/2}$
1	1136·0	88028	$d^8s\ {}^2F_{7/2} - d^8p\ {}^2F^o_{5/2}$
3	1049·8	95256	$d^9\ {}^2D_{5/2} - d^8p\ {}^4F^o_{7\ 2}$
2	1037·4	96395	$d^9\ {}^2D_{5/2} - d^8p\ {}^4D^o_{5\ 2}$
2	1029·9	97097	$d^9\ {}^2D_{5/2} - d^8p\ {}^4F^o_{5\ 2}$

THE RAMAN SPECTRA OF IODIC ACID AND THE ALKALINE IODATES AS SOLIDS AND SOLUTIONS.

By C. S. Venkateswaran.

(From the Department of Physics, Indian Institute of Science, Bangalore.)

1. *Introduction.*

THE study of progressive dissociation of electrolytes in solution by the method of light-scattering was for the first time made use of by I. R. Rao[1] in his investigations on Raman spectra in different concentrations of nitric acid. He has shown that the lines arising from the nitrate ion in solution, first increase in intensity as the concentration is decreased up to a certain point and then become less intense and weak for greater dilutions. In higher concentrations of the acid there are other lines present (some of them fairly strong), which become weaker and weaker and finally disappear as the dilution is gradually increased. It has been pointed out that these lines are to be attributed to the undissociated molecule and that as the dissociation progresses their intensity diminishes while the nitrate lines gain in intensity in a proportionate manner. Thus a comparative study of the Raman effect in different concentrations of an acid provides a beautiful picture of the progress of electrolytic dissociation of its molecules in solution. The method is particularly suitable for concentrated solutions of strong electrolytes for which no other reliable method is available.

Of the systems hitherto studied by this method, nitric acid and sulphuric acid have received the greatest attention, because of their striking and fruitful results. Iodic acid is known to be a strong acid like nitric acid and to contain undissociated molecules even in dilute solutions.[2,3] Its Raman spectrum has been studied, but not in sufficient detail, and the results obtained by the previous investigators[4,5,6,7,8] are found to be discordant

[1] Ramakrishna Rao, I., *Proc. Roy. Soc.*, (A), 1930, **127**, 279.
[2] Kraus and Parker, *Jour. Amer. Soc.*, 1922, **44**, 2429.
[3] Murray and Hartley, *Proc. Roy. Soc.*, (A), 1929, **126**, 84.
[4] Krishnamurti, P., *Ind. Jour. Phys.*, 1930, **5**, 633.
[5] Raman Nayar, M., and Premanath Sharma, *Zeit. f. anorg. u. allg. Chem.*, 1934, **220**, 169.
[6] Nisi, *Jap. Jour. Phys.*, 1929, **5**, 119.
[7] Dickinson and Dillon, *Proc. Nat. Acad. Sci.*, 1929, **50**, 334.
[8] Woodward, L. A., *Phys. Zeits.*, 1931, **32**, 690.

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and their conclusions appear to be based upon incomplete data. Comparatively little attention has also been paid in the past to the study of the Raman effect in iodates. The present investigation was undertaken with a view to clarify the position and obtain a complete Raman spectrum of the acid and its salts which would throw some light on their structure and chemical constitution.

2. *Experimental Details.*

The experimental arrangement that was made use of in the following investigation was the same as that described by the author in a previous communication.[9] In order to keep the alignment of the tube and the spectrograph undisturbed, the apparatus was so designed that the solution could be changed without altering the position of the tube. The Fuess glass spectrograph used in the investigation had a dispersion of about 20 A.U. per millimetre in the 4358 A.U. region and gave satisfactory spectra.

The substances used were supplied by Kahlbaum and marked "analytical". They were further purified by two or three crystallisations. Lithium iodate was prepared by the neutralisation of lithium hydroxide by iodic acid and crystallised out of the solution. Potassium bi-iodate was available only in the state of fine powder and it was used as such without further purification. In spite of the fact that the sodium and lithium iodates as well as the potassium bi-iodate were in a state of fine powder, reasonably good pictures were obtained. The best spectrum, however, was obtained with crystals of less than two millimetres each way in size. Larger crystals were not suitable for the reason that the reflection from the surfaces became greater and increased the intensity of the exciting line, thus rendering the spectrum over-exposed. The spectrograms were taken on Ilford Hypersensitive Panchromatic plate II and D 2500 and measured by means of a Hilger wavelength micrometer. The wavelengths of the modified lines were calculated by linear interpolation from the nearest known lines in the iron arc comparison spectrum.

3. *Results.*

Typical photographs of the spectra obtained are reproduced in the accompanying Plates. The scattered spectra of iodic acid and of the iodates were fairly intense. The acid was examined both in the state of small crystals and in solutions of a wide range of concentrations varying from 18 N to 0·15 N. The best solid picture was obtained at an exposure of about two hours; but plates were also taken at exposures of six and twelve hours in order to bring out some of the fainter lines more prominently. Several

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[9] Venkateswaran, C. S., *Proc. Ind. Acad. Sci.,* (A), 1935, 1, 850.

exposures were made with the solid at different stages of crystallisation in order to make sure that the lines obtained were genuine. The exposure time for the most concentrated solution was two hours and the exposures for the other concentrations were increased in proportion to the dilution. The results obtained for the crystalline solid along with the visual estimates of intensity are given in Table I and those for the solutions are given in

TABLE I.

Iodic acid crystal.

Exciting line	Raman lines		Intensity	Others	
	ν	$\Delta\nu$ in cm.$^{-1}$		Krishnamurti[4]	Nayar and Sharma[5]
4358·3 A.U.	21541	1397	0		
$\nu = 22938$	21689	1249	0		
	22096	842	2		
	22115	823	0		
	22137	801	0		
	22156	782	10	780.7 st.	799.15 m.
	22200	739	1		
	22226	713	8	712.6 v. st.	757.8 st.
	22268	670	0		
	22286	652	0		
	22306	633	6	633 st.	610.6 w.
	22525	413	1		
	22561	377	3		
	22610	328	6	327 m.	473.75 w.
	22624	314	0		
	22643	295	1		
	22718	220	1		262.78 v. w.
	22740	198	0		
	22775	163	0		
	22810	128	1		
	22823	115	0		
	22842	97	0		
	22858	80	1		
	22875	64	1		

A dash above some of the frequency numbers indicates that the anti-stokes of those lines were also present. The letters included within the brackets against some of the numbers in this table as well as in the subsequent ones indicate the strength of the line, *viz., st.* = strong, *v. st.* = very strong; *m.* = medium strong; *w.* = weak; *v. w.* = very weak; *br.* = broad; *v. br.* = very broad; *d.* = diffuse; and *sh.* = sharp.

Table II. The frequency shifts for the iodates of lithium, sodium and potassium are given in Table IV and those for the bi-iodate of potassium in Table V. The Raman lines are in general obtained by 4046, 4358, and

A2   F

TABLE II.

Iodic acid solutions (aqueous).

Exciting line 4358·3 A.U. Raman frequencies in cm.[-1]

18 N	12 N	6 N	4·5 N	3 N	1·5 N	0·5 N	0·15 N
1392 w.	1392 v. w.	..	..	..	..	..	..
1238 w.	1238 v. w.	..	..	..	..	..	..
823 st.	823 st.	823 m.	823 m.	823 m.	819 w.	819 v. w.	..
779 v. st.; v. br.	783 v. st.; v. br.	789 v.st.;br.	789 st. br.	796 st.	796 m.	800 v. w.	..
..	..	796 st.	796 st. sh.	798 st. sh.	799 st. sh.	802 m.sh.	802 w. sh.
644 m.br.	644 st. br.	644 m. br.	644 m. br.	644 m.	644 w.	644 v. w.	..
463 m.	463 w.	463 v. w.	..	..	..	..	..
334 st. ; v. br.	334 v. st.; v. br.	330 st. br.	330 m. br.	328 w.	328 v. w.	328 v. w.	..

TABLE III.

Comparison table. Raman frequencies in cm.[-1]

Author 18 N	Nisi[6] 0.6 N	Dickinson and Dillon[7] 2.7 N	Woodward[c] 10 N—0.8 N	Nayar and Sharma[5] 5 N
1392 w.				
1238 w.				
823 st.				
779 v. st.; v.br.	805 st. 799 m.	800 st.	796 v. st.	821.68 v. st.
644 m.br.	..	..	649 st.	659.01 w.
463 m.		..	..	..
	393 w.			
334 st.; v. br.	317 w.	329 m.	335 m.	358.05 w.

5461 radiations of the mercury arc; but only the values obtained for the 4358 A.U. as exciting line, have been given in the tables. The shifts obtained with the other two lines are used as check on the values given.

The crystalline acid has yielded a number of lines many of which have not been reported previously. The frequency shifts obtained for the strong lines in the solid are in good agreement with those given by Krishnamurti.[4]

TABLE IV.   *Alkali Iodates.*

Substance	Exciting line	Raman lines		Intensity	Others	
		ν	$\Delta\nu$ in cm.$^{-1}$		Krishna-murti, $\Delta\nu$ in cm.$^{-1}$	Nayar & Sharma $\Delta\nu$ in cm.$^{-1}$
KIO$_3$ (crystal)	4358·3 $\nu=22938$	22130	808	medium	808 m.	807.5 m.
		22149	789	medium	784 m.	..
		22185	753	very strong	751.4 v.st	
		22201	737	very strong	733 st.	759 st.
		22598	341	weak		
		22603	335	weak		
		22630	308	weak		
		22806	132	very weak & broad		
		23677	739	weak		
		23691	$\overline{753}$	weak		
		23726	788	very weak		
		23745	$\overline{807}$	very weak		
KIO$_3$ (soln.) (0·25 N at 30°C.)	4358·3 $\nu=22938$	22143	795	strong		
		22616	322	very weak		
KIO$_3$ (soln.) (1.0 N at 90°C.)	,,	22135	803	strong		
		22605	333	weak		
		23740	802	very weak		
NaIO$_3$ (solid)	4358·3 $\nu=22938$	22129	809	medium		
		22151	787	weak		
		22200	738	strong		
		22218	720	very weak		
NaIO$_3$ (soln.) (0.25 N at 30°C.)	,,	22142	796	strong		796 st.[7]
		22614	324	very weak		

Substance	Exciting line	Raman lines		Intensity
		ν	$\Delta\nu$ in cm.$^{-1}$	
LiIO$_3$ (solid)	4358·3 $\nu=22938$	22125	813	weak
		22139	799	strong
		22157	781	medium
		22173	765	very strong
		22330	608	very weak and broad
		22479	459	weak and broad
		22606	332	weak
		22629	309	strong
		22695	243	medium
		22769	169	weak
LiIO$_3$ (soln.) 3.4 N at 30°C.	,, ,,	22144	794	very strong
		22335	603	very weak and broad
		22515	423	very weak and broad
		22618	320	strong
LiIO$_3$ (soln.) 0.25 N at 30°C.	,, ,,	22141	797	strong
		22618	320	very weak

The plate shows the remarkable change that takes place in the spectrum as soon as it is dissolved in water. The sharp lines in the crystal are replaced in the solution by very broad bands which tend, however, to sharpen again as the concentration is decreased.

Table III gives a comparison of the results obtained by the author with those given by the previous investigators. It can be seen that some of the lines in the highest concentrations as well as certain anomalies in intensity and position of the lines observed by the author have not been obtained by the earlier experimenters. This is obviously due to the fact that they have

TABLE V.

Substance	Exciting line	Raman lines		Intensity
		ν	$\Delta\nu$ in cm.$^{-1}$	
Pot. bi-iodate	4358·3 $\nu=22938$	21546	1392	very weak
		21695	1243	very weak
		22120	818	medium, sharp
		22165	773	strong, sharp
		22199	739*	weak, broad
		22305	633	weak
		22621	317	weak
Pot. bi-iodate (solution)	,, ,,	22141	797	strong

worked only with concentrations less than that at which these lines appear. Woodward[8] alone has worked with a 10 N solution in which 463 should have appeared very faintly. The single broad band at about 800 has been identified for the first time as a close doublet consisting of a broad band with the centre at 779 and a comparatively narrow one at 823. The author also has been unable to confirm the line 393 reported by Nisi[6] in his 10% solution. The results given by Nayar and Sharma,[5] both for the crystal and for solution, show large differences from those obtained by the author as well as by others and are evidently due to considerable errors in their measurements.

4. Evidence for Electrolytic Dissociation in Iodic Acid.

Figure 1 gives a schematic representation of the transition that takes place in the spectra of iodic acid when it goes from the state of solid to that of solutions of varying concentrations. The intensity of the Raman lines is represented approximately by the height of the dark lines. It can be seen from the figure that a direct correlation exists between the spectra of the solid and solutions. The whole series of spectra can be explained on the

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* This is the frequency shift of the centre of the band which has a width of about 60 wave numbers.
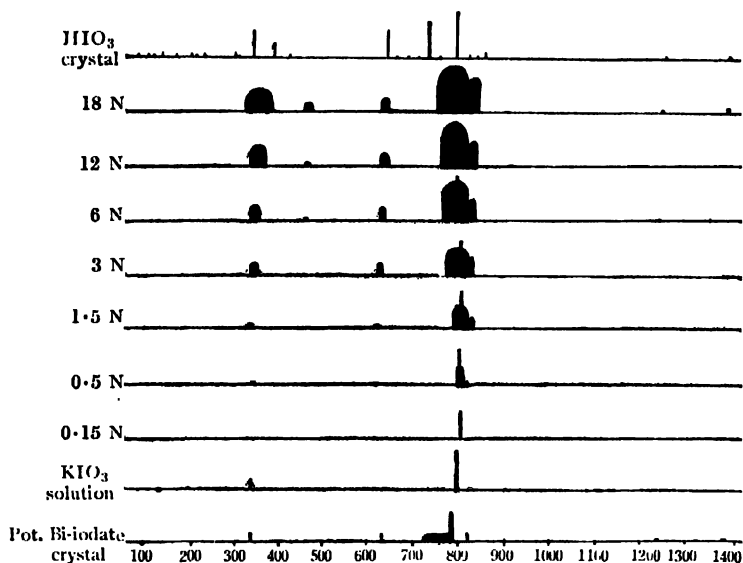
Fig. 1.

assumption that the crystalline acid consists of undissociated HIO_3 molecules and that the dissociation which is very small in concentrated solutions goes on gradually increasing as the concentration diminishes. Thus the sharp and intense lines of the acid at 782, 713, 633, 377 and 328 are due to the vibrations of the HIO_3 molecule. The two lines at 782 and 713 shift slightly to a higher frequency and broaden out, the two broad lines together thus appearing as a single broad band with the maximum at 779 and extending from about 730 to 815. In a similar way the lines at 377 and 328 give rise to the band at 334 covering both the lines. The band at 644 arises from the single line at 633 and is narrower than the other two as can be expected. As the concentration falls off, the number of undissociated molecules decreases and these bands gradually lose both in intensity and in breadth (Fig. 2). The band at 779 appears also to shift in position. The two lines at 1397 and 1249 which are present in the solid and in the 18 N solution do not appear in any other concentrations. There is also a band at 463 in the 18 N solution which diminishes in intensity very rapidly and is practically non-existent at about 4·5 N. These three lines are also to be attributed to the HIO_3 molecule.

At about a concentration of 6 N a fairly sharp line at 796 appears superposed on the band in that region and this goes on increasing in intensity relative to that of the band as the dilution increases. At about 0·5 N this is the strongest line in the spectrum as can be seen from Fig. 4 and the superposed

band is very weak. This line appears also very prominently in the solutions of alkali iodates and is evidently to be attributed to the IO_3 radical. The relative increase in the intensity of this line in the more dilute solutions indicates the progress of dissociation of the acid itself. The other line at 320 observed in the iodate solution is presumably superposed on the HIO_3 band at 334 ; but its effect on the band is too feeble to be observed. The fact that the band at about 800 persists even in solutions of about 0·5 N shows that the dissociation is incomplete even at such high dilutions.

5.   *Evidence for the Polymerisation of the Acid.*

A large mass of chemical evidence[10,11] goes to show that iodic acid is polymerised to an appreciable extent in aqueous solutions ; but the existence of a few contradictory chemical facts keeps it still an open question. In their investigations on the Raman effect, Nayar and Sharma[5] have failed to elicit the band at 634 and 334 in very dilute solutions (0·2 N) and they, there-fore, have pointed out that these two lines are due to the polymers of the acid. But in the investigations of the author these lines appear, though only feebly, in all dilutions and hence the conclusions of the above authors based upon this fact are not justified. There is, however, other evidence to show that I_2O_6'' ions exist in aqueous solutions. As can be seen from Fig. 1, the line at 823 which appears very prominently as a component to the strong band at 779, does not exist either in the solid iodic acid or in the spectrum of the iodates, but is present as a line of medium intensity in the bi-iodate of potassium. This line is, therefore, a positive proof for the exist-ence of I_2O_6'' ions in iodic acid solutions. The intensity of this line goes on diminishing as the dilution increases, which shows that the polymerisation becomes gradually less. The faint appearance of this line even at a concen-tration of 0·5 N indicates that I_2O_6'' ions are present even in dilute solutions.

Though, generally, the Raman lines in the solutions of electrolytes are broader than in the solid which is attributed to the increased freedom of rota-tion of the ions in the solutions, the extraordinary breadth of these lines in the iodic acid solutions is unaccountable unless it is also attributed to the influence of the polymerised molecules themselves. This suggestion is justi-fied by the fact that in the similar case of water, the Raman line correspond-ing to the fundamental oscillation of the molecule appears as a broad band consisting of three components, which has been shown by elaborate analysis[12] to be due to the highly polymerised state of the water molecule.

10 Gmelin's *Handbuch der Anorganische Chemie*, 1933, **8**, 419 J.

11 Raman Nayar, M., and Gairola, T. R., *Zeit. f. anorg. u. allg. Chem.*, 1934, **220**, 163.

12 Ramakrishna Rao, I., *Proc. Roy. Soc.*, (A), 1934, **145**, 489.

In order to examine whether the spectrum of the salt of the dibasic iodic acid shows any similarity to that of the solid or of the concentrated solution, the Raman spectrum of potassium bi-iodate has been investigated both as solid and solutions and the results are given in Table V. The spectrum of the solid resembles, in a general way, that of the iodic acid crystal. But there is the appearance of a line of medium intensity at 818 which is to be attributed to the I_2O_6 radical. The strong line at 713 in iodic acid has spread out into a band with its centre at 739 in the bi-iodate. This fact is very significant for two reasons. Firstly, it shows the effect of polymerisation on the line belonging to the HIO_3 molecule and substantiates our assumption regarding the finite width of bands in the acid solutions. Secondly, it is interesting because, usually crystals give only sharp lines and the appearance of this band in the solid is surprising. The solution of this salt (less than $0 \cdot 1$ N) has given only one line at 797 corresponding to the intense line of the IO_3 radical. From these we are led to conclude that while in the solid the acid salts of the iodic acid are complex compounds in which I_2O_6'' ions exist, in the aqueous solutions, the bi-iodates behave in the Raman effect, as if they are only mixtures of the acid and the alkali iodate.[13]

6. Structure of Iodates.

The fundamental vibrations of the molecules of the AX_3 type have been treated by Dennison[14], assuming a symmetrical pyramidal model. For this type, it has been shown that there are four normal modes of vibration, two of the normal vibrations with frequencies ν_1 and ν_2 being parallel to the axis of symmetry and the other two normal vibrations with frequencies ν_3 and ν_4 being perpendicular to the axis of symmetry (which are doubly degenerate). In the Raman effect ν_1 and ν_2 would appear stronger than ν_3 and ν_4 because the former are optically less active than the latter and ν_1 in which the X atoms execute symmetrical expansions and contractions in their plane while the A atom keeps vibrating along the symmetry axis, would come out as the most intense line. Following the ideas of Hund[15] it was later on shown by Dennison and Hardy[16] that in the case of the pyramidal molecule of the AX_3 type like NH_3, in which the A atom lies close to the plane of the X atoms the parallel vibrations ν_1 and ν_2 become double, the magnitude of the doublet separation depending generally on the height of the pyramid and on the frequency. If the A atom lies in the plane of the X atoms, that is, if the model is coplanar as in the case of the CO_3 or NO_3 radical, the splitting of the

13 Walden, P., *Zeit. Phys. Chem.*, 1888, **2**, 64.
14 Dennison, *Phil. Mag.*, 1926, **1**, 195.
15 Hund, F., *Zeit. f. Phys.*, 1927, **43**, 805.
16 Dennison and Hardy, *Phys. Rev.*, 1932, **39**, 938.

symmetrical oscillations do not take place and the totally symmetrical oscillation which does not involve any change in the electric moment, is optically inactive and appears very strongly in the Raman effect, while the other symmetrical oscillation perpendicular to the plane does not appear at all in the Raman effect. The splitting of the vibrational levels can also occur if there are accidental degeneracies among the frequencies as in the case of CCl_4, CS_2, CO_2, etc.

In the Raman spectra of the iodates we have found that one of the parallel vibrations splits up into a quadruplet and the other into a doublet or triplet and their probable significance will be discussed here.

Table IV gives the Raman frequencies observed for the iodates of lithium, sodium and potassium both in the state of crystals and solutions. A concentrated solution (3·4 N) of lithium iodate has yielded a very strong line at 794 and a fairly strong line at 320 and two weak lines at 423 and 603. In the crystals, all the three iodates give a strong group of four lines in the neighbourhood of 800 and another less prominent group at about 320. Lithium iodate shows also two broad bands at 459 and 608, which probably consist of unresolved components. Besides in the crystals of lithium iodate and potassium iodate as well as in the crystals of iodic acid, there exist a number of lines of lower frequency and these will be treated separately in the succeeding section. These four lines which occur in the iodates as also in the iodic acid evidently belong to the fundamental vibrations of the IO_3 group. Since all the four frequencies appear and both the parallel vibrations exhibit splitting, we are led to conclude that the IO_3 radical is pyramidal in structure with the I atom close to the plane of the O atoms. In such a case the two groups of lines at about 800 and 320 correspond to ν_1 and ν_2 respectively and the two faint lines at 608 and 459 correspond to ν_3 and ν_4 respectively. The infra-red data for potassium iodate gives the absorption frequencies at 781, 748 and 371.[17,18]

Though the relative shifts and intensities of the components differ widely from one component to another, the differences between the first two lower shifts and the last two higher shifts are almost equal and are of the same magnitude from compound to compound. These four components can be represented by the relation $\nu_1' = \nu_1 \pm \alpha \pm \beta$, where ν_1 is the average of all components, β is half the difference between one and two or three and four and α is half the difference between one and three or two and four. Table VI gives

17 Schaefer and Schubert, *Ann. der Phys.*, 1921, **7**, 309.

18 Laski, *Zeits. f. Krist.*, 1927, **65**, 607.

the values of ν_1, α and β and the calculated values of frequencies together with the observed shifts of the components.

TABLE VI.

Substance	ν_1	α	β	Calculated				Observed			
				$\nu_1+\alpha+\beta$	$\nu_1+\alpha-\beta$	$\nu_1-\alpha+\beta$	$\nu_1-\alpha-\beta$	1	2	3	4
HIO₃	812	8·5	20·5	841	800	824	783	842 w.	801 v.w.	823 v.w.	782 st.
LiIO₃	789	7	16	812	780	798	766	813 w.	781 m.	799 st.	765 v.st.
NaIO₃	764	10	35	809	739	789	719	809 m.	739 st.	787 w.	720 w.
KIO₃	770	10	26	806	754	786	734	808 m.	753 v.st.	789 m.	737 st.

A tentative explanation for this interesting fact is given as follows. The vibrational energy levels with frequency ν_1 is split up in the first instance, to a doublet in accordance with the Hund-Dennison theory and is further split up, to form a quadruplet due to an accidental degeneracy $\nu_1 - \nu_2 + \nu_4$. In the case of lithium iodate where four frequencies are observed, this condition has been satisfied. That the parallel vibration with frequency ν_2 also shows splitting in all cases lends support to our postulate regarding the first split of ν_1. If the above explanation is valid, the height of the pyramid should be small enough to cause the splitting and the X-ray evidence[19, 20] seems to support it.

It should be remarked that the relative shifts and intensities of the components differ from compound to compound as illustrated in Fig. 5 and this is to be explained as due to the influence of the cation on the vibrations of the radical. That the cation in the solid aggregate has a great influence on the vibrations of this radical, is also demonstrated by the fact that in the solutions of all the iodates where the influence of the metal ion is considerably less, the components vanish giving place to strong lines of finite breadth. The vibration frequencies of lithium, sodium and potassium show also a small increase according to the increasing atomic weight. The strong line of lithium iodate likewise, appears to shift towards lower frequencies as the concentration increases. The effect of temperature on the main vibration frequency appears to be opposite to that of concentration as can be seen from the values given in Table IV, for potassium iodate solution at 30°C.

[19] Zachariasen, Z. Krist., 1929, 71, 501 and 517.
[20] Zachariasen and Barta, Phys. Rev., 1931, 37, 1626.

and 90°C. A detailed theoretical analysis of the spectra of the iodates is deferred to a later publication.

7. *Low Frequency Oscillations.*

A clear spectrum of the crystals of iodic acid shows besides the lines corresponding to the molecular structure described above, a number of lines with a maximum shift of about 220 (Fig. 6) in the neighbourhood of the exciting line, namely, 220, 198, 163, 128, 115, 197, 80 and 64. The antistokes of some of these lines are also faintly visible, but could not be measured because of the presence of the companions for the exciting line. Two similar low frequency shifts at 243 and 169 are also obtained for solid lithium iodate (Fig. 7) and only one at 132 for the potassium iodate crystals. These lines are completely washed out in solutions and there is an appearance of faint wings on either side of the exciting line. Similar low frequency oscillations have been observed previously by the earlier investigators in some crystals of carbonates,[21] and nitrates[22] and quite recently by Gross and Vuks[23] in the crystals of diphenyl, benzene and naphthalene and are explained as belonging to the lattice vibrations in the crystal. Thus, one possible explanation for these observed low frequency oscillations in the iodates is that they are connected with the vibrations of the layers of the atoms in the crystal lattice against each other. But it is hard to believe that the lattice is capable of oscillations giving rise to such a large number of lines as is the case with iodic acid. Another alternative explanation that may be put forward is on the lines suggested by Bhagavantam,[24] namely, that these low frequency oscillations are due to the oscillational motions or restricted rotations of the molecules in the solid state. The molecules possess greater freedom for rotation in solution and exhibit the phenomenon of wings. The whole theory of the so-called lattice vibrations is, however, in its infancy and no more can be said about them until a clearer picture of it is forthcoming.

In conclusion, the author wishes to express his grateful thanks to Prof. Sir C. V. Raman, F.R.S., N.L., for his helpful interest in the work. The author's thanks are also due to Dr. P. Krishnamurti for his keen interest in the work.

Summary.

The Raman spectrum of the iodic acid as a function of concentration and of the iodates of lithium, sodium and potassium in the state of the solid and solutions have been investigated. The crystals of iodic acid yield five

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[21] Bhagavantam, S., *Zeits. fur Kryst.*, 1931, **77**, 43.

[22] Cabannes and Canals, *Compt. Rend.*, 1931, **193**, 289.

[23] Gross and Vuks, *Nature*, 1935, **135**, 100; *Nature*, 1935, **135**, 431.

[24] Bhagavantam, S., *Ind. Jour. Phys.*, 1933, **8**, 197; see also Pauling, L., *Phys. Rev.*, 1930, **36**, 430.

intense lines and a number of weak and sharp lines, which are replaced by intense and broad bands in solution. The spectrum of the solutions for a very wide range of concentrations varying from 18 N to 0·15 N show an anomalous behaviour regarding intensity and frequency shifts. This fact has not been observed previously because the earlier authors had not worked with such high concentrations. From a qualitative study of the solid and solutions, evidence has been obtained for the progressive dissociation of the acid. The results show that the dissociation is incomplete even at concentrations of 0·5 N. Suggestions have also been put forward that the acid is polymerised in the solutions to an appreciable extent, the polymerisation decreasing with dilution.

The spectra of the iodates give in general two lines which are identified with the two possible parallel oscillations of the pyramidal form of the molecules of the AX_3 type and in the case of lithium iodate and iodic acid two more lines are obtained corresponding to the two perpendicular oscillations. The main parallel vibrations of the radical are split up into components in all the iodates including iodic acid, the relative shifts and intensities being different from compound to compound, the number of components being four in the case of ν_1 and two or three in the case of ν_2. The splitting of the vibration with the frequency ν_1 has been explained as partly due to the positional degeneracy pointed out by Hund and Dennison and as partly due to the accidental degeneracy, $\nu_1 = \nu_2 + \nu_4$. The shift and the relative intensities of these components are also shown to be influenced by the metal ion. The shift of the intense line in the solution depends to some extent on the cation, on concentration and on temperature.

A large number of low frequency oscillations has also been obtained in the crystals of iodic acid. Two such oscillations occur in the lithium iodate and only one in potassium iodate crystals. An explanation of these lines is briefly suggested.

Potassium bi-iodate crystal has given a spectrum somewhat similar to that of iodic acid. The presence of a new line in its spectrum indicates the existence of I_2O_6'' ions. The existence of a band in the spectrum of the solid is very significant. The solution of this salt behaves as if it were only a mixture of HIO_3 and KIO_3.
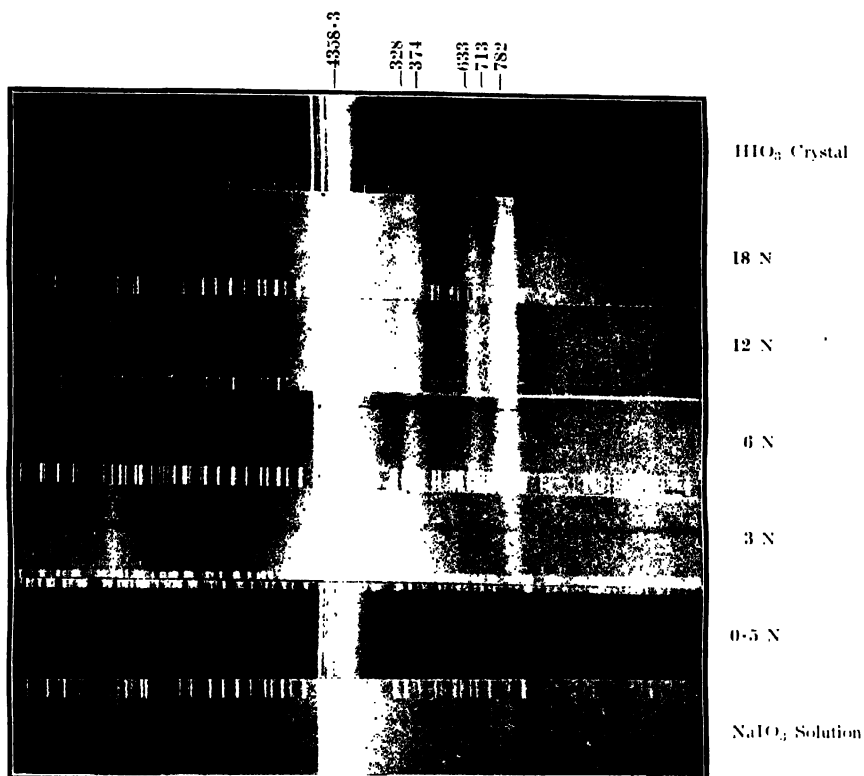
FIG. 2.
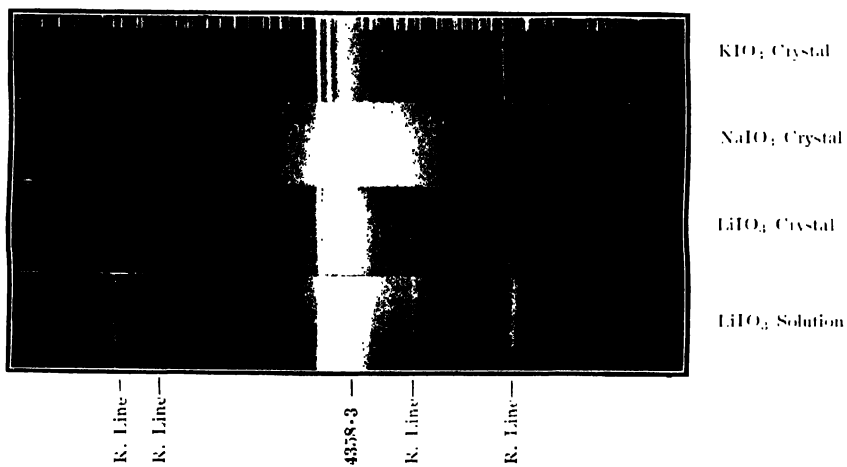
FIG. 3.

FIG. 4. HIO₃ Solutions
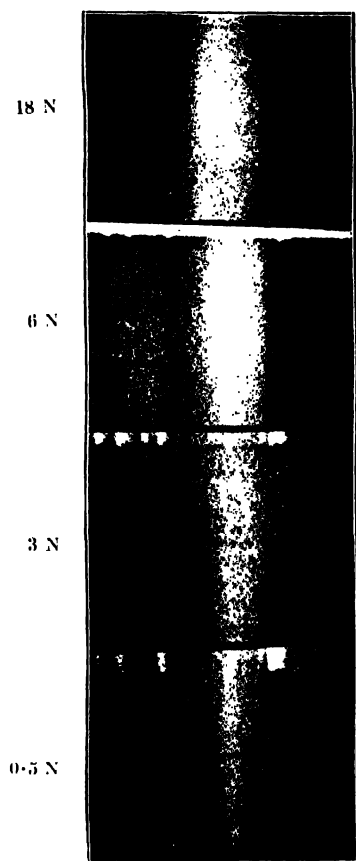
FIG. 5. Iodate Crystals.
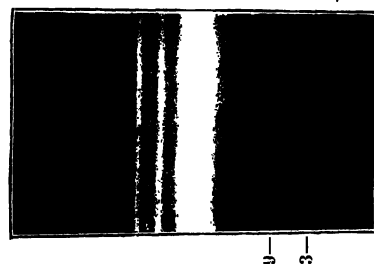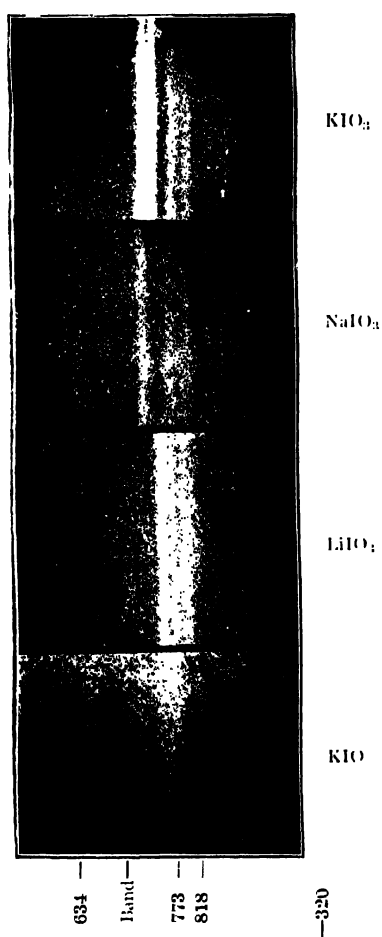
FIG. 6.
HIO₃ Solid showing low frequency oscillations.

FIG. 7.
LiIO₃ Solid showing low frequency oscillations

ON THE CONVERGENCE ERROR IN DEPOLARISA-
TION MEASUREMENTS.

BY R. ANANTHAKRISHNAN.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. *Introduction.*

So much importance attaches to the precise measurement and interpreta-
tion of the degree of depolarisation of scattered light—be it in Tyndall,
Rayleigh or Raman scattering—that no apology is needed for a paper deal-
ing with a somewhat difficult point arising in the technique of such measure-
ments, namely, the errors due to the lack of parallelism in the rays of light
entering the medium and scattered by it. In practice it is not possible to
realise the theoretical ideal of the illumination of the medium by a beam of
parallel rays, and the examination of the scattered light in a direction strictly
transverse to the latter. The comparatively feeble intensity of the scattered
light usually entails the use of a source of finite dimensions and of a lens
to concentrate the beam of light during its passage through the medium.
In consequence the rays of light are neither parallel among themselves, nor
strictly perpendicular to the direction of observation, and errors arise which
may assume great importance when considering relatively small depolari-
sations.

There has been considerable divergence of opinion regarding the magni-
tude of the errors arising in the manner referred to above, and particularly
in the case when an illuminating lens of large aperture is used to focus an
image of the source of light within the medium at the point of observation.
It is obvious that the rays starting from a given point on the source and
reaching the conjugate point in the focal plane are optically coherent with
each other. Dr. I. Ramakrishna Rao[1] took this coherence into account
and claimed that no correction is necessary to the observed value of the
depolarisation on account of the finite aperture of the lens provided the
measurements are made precisely at the focus. On the other hand Gans[2]
assumed the rays meeting at the focus to be incoherent—an assumption

[1] I. R. Rao, *Ind. Journ. Phys.,* 1927, 2, 72.
[2] R. Gans, *Physik. Zeits.,* 1927, 28, 661.

obviously not justifiable—and derived the formula

$$\rho = \rho_0 + \tfrac{1}{2} \sin^2 \theta = \rho_0 + \frac{\theta^2}{2}$$

where ρ_0 — depolarisation with parallel unpolarised light

and ρ — observed value of the depolarisation when the incident beam has a semi-convergence θ.

Cabannes[3] has discussed these rival views in his book and attempted to reconcile them, but the question has remained unsettled and somewhat obscure.

2. Case of two Intersecting Parallel Beams.

To elucidate the points at issue it is desirable to treat at first a relatively simple case.

Consider a pair of parallel, coplanar, plane polarised beams crossing each other at an angle 2θ. Let the electric vector in the beams be in the plane of the paper which is supposed to be the xy-plane. The region over which the two beams are superposed will be an interference field, the interference maxima and minima being bands parallel to the x-axis, occurring with a regular periodicity $\frac{\lambda}{4 \sin \theta}$.
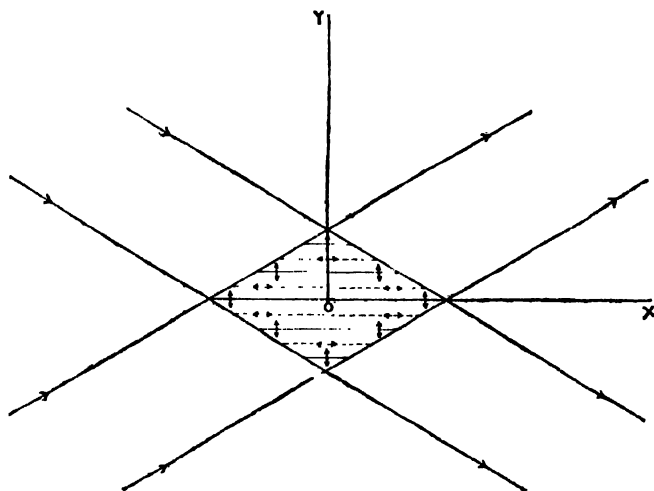
Fig. 1.

At the interference maxima (represented by thick lines in the figure), the x-components of the electric intensity annul each other so that the resultant electric vector is vertical, while the interference minima (represented by dotted lines in the figure) are places where the resultant vibration is parallel

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[3] J. Cabannes, *La Diffusion Moléculaire de la Lumière.*

to the axis of x. If the region of overlapping is occupied by molecules of a gas which scatter light in the usual way, and if one were to make observations in a direction perpendicular to the plane of the paper, *the depolarisation observed along the interference maxima will be the correct value, while at the interference minima, the electric vector being horizontal the depolarisation ratio will be reversed.* Let it now be assumed that on account of some reason, such as for instance, the lack of monochromatism in the interfering beams or the excessive closeness of the interference bands, that it is not possible to concentrate attention on the individual interference maxima. The depolarisation measured in the usual way in such circumstances would be that averaged over the whole field, due regard being had to the intensity, and would thus differ from the correct value.

At any point (x, y) in the interference field where the relative retardation between the interfering beams is $\delta = 2\, y \sin \theta$, the components of the electric intensity are given by

$$\begin{cases} E_x = 2E \sin \theta . \quad \sin (k\, y \sin \theta) \quad \sin k\, (vt + y \sin \theta). \\ E_y = 2E \cos \theta . \quad \cos (k\, y \sin \theta) \quad \cos k\, (vt + y \sin \theta). \\ E_z = 0 \end{cases} \qquad .. \qquad .. \quad (1)$$

where $k = \dfrac{2\pi}{\lambda}$

The time mean square of the components of the electric moment induced in a molecule situated at x, y are given by the equations[4]

$$\begin{cases} \overline{p_x^2} = (A - B)\, \overline{E_x^2} + B\, (\overline{E_x^2 + E_y^2 + E_z^2}) \\ \overline{p_y^2} = (A - B)\, \overline{E_y^2} + B\, (\overline{E_x^2 + E_y^2 + E_z^2}) \\ \overline{p_z^2} = (A - B)\, \overline{E_z^2} + B\, (\overline{E_x^2 + E_y^2 + E_z^2}) \end{cases} \qquad .. \qquad .. \quad (2)$$

where $A = \dfrac{1}{5} \Sigma g_1^2 + \dfrac{2}{15} \Sigma g_1 g_2$

and $B = \dfrac{1}{15} (\Sigma g_1^2 - \Sigma g_1 g_2)$

g_1, g_2, g_3, being the principal polarisabilities of the molecule.

The components of the intensity of the scattered radiation polarised parallel to the x, y and z directions will be obtained by integrating (2) over the entire field. If we call these components $\overline{P_x^2}$, $\overline{P_y^2}$, $\overline{P_z^2}$, then the depolarisation for observation in a direction perpendicular to the plane of the paper is given by

$$\rho = \frac{P_x^2}{P_y^2} = \frac{A \sin^2\theta + B \cos^2\theta}{B \sin^2\theta + A \cos^2\theta} = \rho_0 + \theta^2 \qquad .. \qquad .. \quad (3)$$

where ρ_0 is the depolarisation when $\theta = 0$.

---

[4] R. Gans, *loc. cit.*

The same result follows if the two beams are considered as incoherent and if we adopt the procedure of Gans.

3. Lens with Square Aperture.

We shall next consider the case in which a lens of focal length f limited by a square aperture of edge a is used to concentrate the light on the medium.

Let the plane of the aperture be the xy-plane and let the direction of propagation be the z-axis. Let the incident light before its passage through the lens be polarised with the electric vector parallel to the axis of x, and let the vibrations be given by E cos kvt.

The components of the electric intensity at any point in the focal plane whose co-ordinates are ζ and η due to an element $dx\,dy$ of the aperture whose co-ordinates are x, y, z are given by[5]

$$E_x = -\frac{1}{\lambda f} E \cos \phi . \ \sin k \left(vt - f + \frac{x\zeta + y\eta}{f}\right) dx\,dy$$
$$E_y = 0 \tag{4}$$
$$E_z = -\frac{1}{\lambda f} E \sin \phi . \ \sin k \left(v \quad f + \frac{x\zeta + y\eta}{f}\right) dx\,dy$$

where ϕ is the inclination of the electric vector in the ray from the element with the axis of x.

As a first approximation we may put

$$\cos \phi = 1 \text{ and } \sin \phi = \frac{r}{f}$$

The components of the electric intensity at M due to the whole aperture are obtained by integrating (4) over the entire aperture. They are

$$E_x = -\frac{E}{\lambda f} a^2 \sin k \ (vt - f) \left[\frac{\sin u}{u} . \frac{\sin v}{v}\right]$$
$$E_y = 0 \tag{5}$$
$$E_z = -\frac{E}{\lambda f^2} \frac{a^3}{2} \cos k \ (vt \quad f) . \left[\frac{\sin v}{v}\right] \left[\frac{\sin u}{u^2} - \frac{\cos u}{u}\right]$$

where $u = \frac{\pi a \zeta}{f\lambda}$ and $v = \frac{\pi a \eta}{f\lambda}$.

Substituting these values of E_x, E_y, E_z, in equations (2) of the preceding section, and following the same treatment we get, after putting $\frac{a}{f} = 2\theta$

$$\overline{P_x^2} = \int\!\!\int_{-\infty}^{+\infty} (A\,E_x^2 + B\,\overline{E_z^2})d\zeta d\eta = \tfrac{1}{2} E^2 a^2 \ [A + B\tfrac{1}{3}\theta^2]$$

$$\overline{P_y^2} = \int\!\!\int_{-\infty}^{+\infty} (B\,E_x^2 + B\,\overline{E_z^2})d\zeta d\eta = \tfrac{1}{2} E^2 a^2 \ [B + B\tfrac{1}{3}\theta^2] \tag{6}$$

$$\overline{P_z^2} = \int\!\!\int_{-\infty}^{+\infty} (B\,\overline{E_x^2} + A\,E_z^2)d\zeta d\eta = \tfrac{1}{2} E^2 a^2 \ [B + A\tfrac{1}{3}\theta^2]$$

[5] See Lord Rayleigh, *Scientific Papers*, Vol. III, p. 80.

From these it follows that the depolarisation of the light scattered in the direction Oy is given by

$$\rho_v = \frac{P_z^2}{P_x^2} = \rho_{v_0} + \tfrac{1}{3}\theta^2 \quad .. \qquad .. \qquad .. \qquad .. \qquad (7)$$

where ρ_{v_0} is the value of ρ_v for $\theta = 0$.

If observations are made in the direction Ox, we have

$$\rho_h = \frac{P_z^2}{P_{y'}^2} = 1 + \frac{1}{\rho_{v_0}} \tfrac{1}{3}\theta^2 \qquad .. \qquad .. \qquad .. \qquad (8)$$

If the incident light is unpolarised, it is easily seen that the depolarisation of the transversely scattered light is given by

$$\rho_u = \rho_{u_0} + \tfrac{2}{3}\theta^2 \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad (9)$$

From (7) and (9) it follows that so far as convergence error is concerned, no advantage is gained by using light polarised with the electric vector vertical in place of unpolarised light, since ρ_{v_0} is nearly half of ρ_{u_0}. From (8) we see that when the incident light is polarised with the electric vector horizontal, the effect of convergence is to make the horizontal component appear brighter than the vertical and this effect becomes all the more striking when the value of the depolarisation is very small. Formulæ (7), (8) and (9) also follow readily if the incident beam is treated as incoherent, and if we adopt the method of Gans. What actually takes place is that the finite width of the source such as the sun, for instance, produces a mixing up of the diffraction patterns due to the different points of the source so that the ultimate effect is the same as though the rays from any single point are incoherent.

4. Lens with Circular Aperture.

We now go to consider the case of greater practical interest and importance, namely, the case in which the condensing lens is limited by a circular aperture of radius R.

We follow the treatment already given for the square aperture.. Putting $\frac{k\zeta}{f} = p$ and $\frac{k\eta}{f} = q$, and noting that the form of the aperture is symmetrical with respect to the axes of x and y, the expressions for the electric intensity at any point in the focal plane may be written in the form

$$\begin{aligned} E_x &= -\frac{E}{\lambda f}\sin k\,(vt-f)\iint\cos px\cos qy\,dx\,dy \\ E_z' &= \frac{E}{\lambda f^2}\cos k\,(vt-f)\;\frac{d}{dp}\left[\iint\cos px\cos qy\,dx\,dy\right] \end{aligned} \qquad (10)$$

When the convergence of the incident beam is not too great, it is justifiable as a first approximation to assume that the x-component of the electric intensity is symmetrical with respect to the focal point $p=0$, $q=0$. It is thus sufficient to determine E_x along the axis of p, and so the integral in (10)

reduces to the usual diffraction integral in the case of the circular aperture. We thus obtain for the time mean square of E_x, E_y and E_z

$$
\left.
\begin{aligned}
\bar{E_x^2} &= \frac{E^2}{2\lambda^2 f^2} \left[\pi R^2 \frac{2J_1\left(\frac{2\pi Rr}{f\lambda}\right)}{\left(\frac{2\pi Rr}{f\lambda}\right)} \right]^2 \\
\bar{E_y^2} &= 0 \\
\bar{E_z^2} &= \frac{E^2}{2\lambda^2 f^4} \left[\frac{d}{dp} \left\{ \pi R^2 \frac{2J_1\left(\frac{2\pi Rr}{f\lambda}\right)}{\left(\frac{2\pi Rr}{f\lambda}\right)} \right\} \right]^2
\end{aligned}
\right\} \qquad \qquad \dots \dots (11)
$$

where r is the distance of the point in the focal plane under consideration from the focus $p=0$, $q=0$.

It is readily shown that

$$
\iint_0^\infty E_x^2 \, d\zeta d\eta = \frac{E^2}{2} \pi R^2 2 \int_0^\infty z^{-1} J_1^2(z) \, dz = \frac{E^2}{2} \pi R^2 \qquad (12)
$$

and

$$
\iint_\infty^{+\infty} E_z^2 \, d\zeta d\eta = \frac{E^2}{2} \pi R^2 \frac{R^2}{f^2} \int_0^\infty z^{-1} J_2^2(z) dz = \frac{E^2}{2} \pi R^2 \tfrac{1}{4}\theta^2 \qquad (13)
$$

where $z = \frac{2\pi Rr}{f\lambda}$ and $\theta = \frac{R}{f}$.

Proceeding to find $\bar{P_x^2}$, $\bar{P_y^2}$ and $\bar{P_z^2}$, as in the case of the square aperture we obtain,

$$
\left.
\begin{aligned}
\rho_v &= \rho_{v_0} + \tfrac{1}{4}\theta^2 \\
\rho_h &= 1 + \frac{1}{\rho_{v_0}} \tfrac{1}{4}\theta^2 \\
\rho_u &= \rho_{u_0} + \tfrac{1}{2}\theta^2
\end{aligned}
\right\} \qquad \dots \qquad \dots \qquad \dots \qquad \dots \qquad \dots (14)
$$

The last of the results (11) has been worked out by Gans using the idea of incoherence, while the first two which relate to the cases in which the incident light is plane-polarised can be obtained by adopting the same procedure. The significance of (14) has been already explained in section (3).

5. Remarks on the Foregoing Results.

The above investigation relates to the focal plane and assumes that the lens is uniformly illuminated and that every part of the aperture is equally effective at any given point in the focal plane where there is any appreciable intensity. This requires the use of a lens which is optically perfect. Whether it is so or not can be determined by the well-known knife-edge test. Sunlight reflected by a heliostat is allowed to illumine the entire aperture of the lens, and the rays diverging from the focus are allowed to fall on a distant screen. A sharp razor-blade is slowly made to advance through the focal

plane and the illumination of the circular patch of light on the screen is watched. If the lens is optically good, the illumination of the entire area fades off quite regularly and uniformly, so that by merely looking at the screen it is impossible to find out from which side the knife-edge is cutting across. Actual experiment with a high-class photographic lens shows that even for a quite appreciable distance on either side of the focal plane, the knife-edge test gives the same result, and within this region, the theoretical results as regards the magnitude of the convergence correction would be expected to be very nearly valid. As the extent of this region is quite comparable with the dimensions of the track over which one concentrates in practice in depolarisation work, the theoretical correction can be safely applied to the observed value so long as the lens used in the experiment is optically good, and observations are restricted to the vicinity of the focus. When we deviate far too much from the focal plane, the knife-edge test reveals a decided asymmetry in the illumination at any point, which is shown by the definite appearance of the shadow of the knife-edge marching across the illuminated area on the screen. Under such circumstances the validity of the theoretical treatment would no longer hold good, and the magnitude of the correction at any point will depend upon whether it receives illumination from the central or marginal portions of the lens. The actual correction will be smaller than the theoretical value in the former case, and larger in the latter, and the observed depolarisation of the transversely scattered light will thus vary from point to point within the visible track of the illuminating beam within the medium.

So far the necessity for the use of an optically perfect lens has been stressed as a necessary consequence of the theoretical considerations, and this evidently implies that it is also of the highest importance to have the rest of the optical parts as perfect as possible. If sunlight is employed for depolarisation work, it is essential to use a good heliostat mirror to reflect the light into the observation chamber. To avoid distortion of the incident beam as it enters the vessel containing the scattering medium, it is essential to use a flat window. The use of a bulb is highly unsatisfactory in this respect. It is desirable also that the window through which the scattered track is viewed should be free from optical defects.

6. *Some Experimental Results.*

It has been already remarked that the convergence error becomes very important when the genuine depolarisation of the substance under investigation is very small. This at once suggests that gases and vapours of small depolarisation as well as liquids and liquid mixtures near the critical point are the media in which the depolarisation would be expected to be most

susceptible to the change in convergence of the incident beam. From a practical point of view, however, the intensity of scattering is a very important factor in the experimental investigation of the question, since the accurate determination of the depolarisation over a wide range of convergence is feasible only if the intensity of scattering is relatively large.

(a) *Gases.*—Isobutane seemed to be favourable in the case of gases, as its depolarisation is very small, while the scattering is at the same time relatively intense. The gas was taken from a cylinder supplied by the Ohio Chemical Works, and was guaranteed to be 99% pure. The depolarisation was determined independently using two different arrangements.

(1) The gas was contained in a pear-shaped bulb of the type used by Ramanathan and I. R. Rao in their investigations, but with the addition of a projecting tube with flat window on the observation end. Sunlight reflected by a Foucault heliostat was concentrated at the centre of the bulb using a high class condensing lens in combination with an adjustable iris diaphragm, and the depolarisation of the transversely scattered light was measured in the usual way. The angle of convergence of the incident beam was determined by measuring the diameter of the illuminated area on a screen placed at a distance of one metre from the focus. The results are given in the table below :—

TABLE I.

Convergence of the incident beam $= 2\theta$	Observed value of the depolarisation $= \rho_u$	Correction $\theta^2 = \dfrac{\theta^2}{2}$	Corrected value of the depolarisation $= \rho_{u_0} = \rho_u \dfrac{\theta^2}{2}$
Deg. Radian			
$29°\ 18' = 0\cdot520$	$4\cdot04\%$	$3\cdot38\%$	$0\cdot66\%$
$19°\ 48' = 0\cdot346$	$2\cdot27\%$	$1\cdot45\%$	$0\cdot82\%$
$9°\ 54' = 0\cdot173$	$1\cdot06\%$	$0\cdot37\%$	$0\cdot69\%$

Mean value $= 0\cdot72\%$

The values in the last column show that, in spite of the large value of the observed depolarisation when the convergence of the incident beam is large, the corrected value comes out to be very nearly the same as that for small values of convergence.

(2) The gas was contained in a cross made of pyrex glass with fused-on end plates and suitable diaphragms. A Dallmeyer photographic lens of

focal length 1 foot and adjustable aperture was used for concentrating the sunlight at the centre of the cross. The optics of the whole arrangement was thus much superior to that used in the first case. The results are recorded in the table below.

TABLE II.

Aperture	ρ_u	$\dfrac{\theta^2}{2}$	$\rho_{u_0} - \rho_u - \dfrac{\theta^2}{2}$
F/5·6	0·83%	0·4%	0·43%
F/11	0·58%	0·1%	0·18%

Mean value = 0.46%

The difference in the values of the depolarisation measured with the bulb and with the cross, emphasises the necessity for the perfection of the optical parts used in depolarisation work.

(b) *Liquids.*—A critical mixture of methyl alcohol and hexane distilled and sealed in a spherical bulb was found to be specially suited for the study of the convergence correction in the case of liquids, because of the close proximity of its critical temperature (29° C.) to the room temperature. The mixture was warmed just above the critical point of complete miscibility and was placed in the path of the incident beam in such a way that the centre of the bulb coincided with the focus. The use of a spherical bulb as stated above eliminates the alteration in the convergence of the track which would otherwise require to be considered. The observed and corrected values of the depolarisation are given below.

TABLE III.

2θ		ρ_u	$\dfrac{\theta^2}{2}$	$\rho_{u_0} = \rho_u - \dfrac{\theta^2}{2}$
Deg.	Radian			
28° 20′	=0·4945	3·34%	3·05%	0·29%
18° 11′	=0·3173	1·49%	1·26%	0·23%
4° 35′	=0·08	0·44%	0·08%	0·36%

Mean value = 0·29%

The observation of the Tyndall cone through a nicol in the case of the liquid mixture reveals certain interesting features which illustrate the

theoretical discussion contained in section (5). Using an incident beam of large convergence, when the nicol through which the transversely scattered light is viewed is rotated so as to transmit only the horizontal component, one finds a dark region in the middle of the Tyndall cone on either side of the focus, while the margins appear as a pair of bright streamers (Fig. 3 in the Plate). A slight rotation of the nicol either way makes the luminous cone asymmetric, the intensity fading off rapidly from one edge to the other (Figs. 2 and 4 in the Plate). The explanation for these appearances is almost self-evident in view of what has been stated in section (5). Fig. 3 in the Plate brings out in a picturesque fashion the whole mechanism of the convergence error. The dark centre shows how the genuine depolarisation is exceedingly small, and how the greater part of the illumination observed at the focus is contributed by the marginal portions of the lens.

In conclusion, it is my greatest pleasure to record my respectful thanks to Professor Sir C. V. Raman, for suggesting the present investigation, and for much valuable guidance and criticism in the course of the work.

7. Summary.

The scattering of light in an interference field is discussed, and it is shown that for the simple case of two parallel plane-polarised intersecting beams, the depolarisation at the interference maxima gives the correct value, while at the interference minima the depolarisation ratio is reversed. The average of the depolarisation taken over the whole field is higher than the correct value. The treatment is extended to the cases in which a lens covered with a square aperture, and with a circular aperture, respectively, is used to concentrate the light on the scattering medium. It is shown that the observed values of the depolarisation would deviate from the genuine values by a correction factor which involves the square of the angle of convergence. The observed depolarisation ρ is given by

$$\rho_v = \rho_{v_0} + a\theta^2$$
$$\rho_h = 1 + \frac{1}{\rho_{v_0}} a\theta^2$$
$$\rho_u = \rho_{u_0} + 2a\theta^2$$

where a has the value $\frac{1}{3}$ for a square aperture, and $\frac{1}{4}$ for a circular one. The subscripts v, h, u refer to the cases in which the incident light has its electric vector vertical, horizontal and is unpolarised respectively. It is pointed out that the same results follow by treating the incident beam as a bundle of incoherent rays. Some consequences of the theoretical results are discussed and the necessity for the perfection of the optical parts used in depolarisation work is emphasised. Experimental results are given which illustrate the points discussed in the paper.

FIG. 2.

FIG. 3.

FIG. 4.

THE DYNAMICAL THEORY OF THE DIAMOND LATTICE.

Part III. The Diamond-Graphite Transformation.

BY N. S. NAGENDRA NATH.

(From the Department of Physics, Indian Institute of Science, Bangalore)

1. Introduction.

IT is common knowledge that diamond is spontaneously transformed to graphite or black carbon at high temperatures. There have been many investigations to find out whether the transformed substance is really graphite or contains any other modification of carbon and to determine the exact temperature of transformation of diamond. That the transformed substance consists of minute crystals of graphite has been established by two Hungarian investigators Emerich Szarvasy and Béla Lányi.[1] They found that the transformation was rapid at 1760° C. but unchanged diamond was still present. On Röntgen examination, they found that the relative intensities of the stronger graphite lines are the same in natural or artificial Acheson graphite and in graphite formed from diamond by graphitization. The size of the sub-microscopic crystals of graphite formed from diamond at 2000° C. was about the same as those of graphite produced from methane. G. Friedel and G. Ribaud[2] who have studied the weak birefringence in diamond have also studied the transformation of diamond to graphite and they find the temperature of transformation to be 1885° C. P. Libeau and M. Picon[3] have also studied the transformation with their technique of high temperature heating and they place the temperature of the rapid transformation at about 2000° C. At lower temperatures they find that the transformed substance consists not only of graphite but also of black diamond which gives the same X-ray pattern[4] as diamond itself. M. de Kay Thompson and P. K. Frölich[5] have also studied the conversion of diamond to graphite

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[1] Emerich Szarvasy and Béla Lányi, *Math. Naturw. Anz. Ungar. Akad. Wiss.*, 1931, **48**, 137.

[2] G Friedel and G. Ribaud, *Comptes Rendus*, 1924, **178**, 1126.

[3] P. Libeau and M. Picon, *Comptes Rendus*, 1924, **179**, 1059.

[4] W. Gerlach, *Zeit. f. Anorg. und Alg. Chemie*, 1924, **137**, 331.

[5] M. de Kay Thompson and Per K. Frölich, *Trans. Amer. Electro-chem. Soc.*, 1923, **43**, 161.

at various temperatures for various times of heating confirming that the transformed substance is graphite by Brodie's Test. They place the temperature of slow transformation at 1650° C. and rapid transformation at 1750° C. and remark that diamonds turn black at 1200° C. due to the numerous cracks causing the absorption of light by total reflection. If this explanation is genuine, it seems to offer also an explanation for the blackening noticed by P. Libeau and M. Picon at such temperatures. The conclusion of Vogel and Tamman[6] who have studied the conversion of diamond, that diamond starts converting to graphite at 1000° C. appears to require modification in the light of the remark of M. de Kay Thompson and P. K. Frölich. There are many others who have studied the transformation from the time of Francis I who burnt a portion of diamond at the focus of a large concave mirror. Among them are Jacquelain,[7] v. Schrötter,[8] Moisson,[9] Rose[10] and Hon. C. A. Parsons and A. A. C. Swinton.[11]

The purpose of this paper is to explain as to how the graphite structure can be derived from that of diamond by a simple transformation and also to calculate the temperature at which diamond transforms to graphite. The calculated temperature is in satisfactory agreement with the experimental determinations. The ideas followed up here seem to be not very special to the diamond-graphite transformation itself but can have extensions for similar types of transformation of other substances.

2. *The Diamond-Graphite Transformation.*

It has been shown by X-ray analysis that the diamond structure[12, 13] is made up of two cubic face-centred lattices interpenetrating one another in a manner such that an atom of one lattice is equally nearest to four atoms of the other lattice, the C-C distance being 1·54 Å and the edge of the unit cubic cell being 3·56 Å. Bernal,[14] Hassel and Mark[15] and others have shown that the graphite structure is made up of series of parallel plane equidistant layers of carbon atoms and that each layer is a hexagonal net of atoms such that half the atoms in a layer lie directly along the lines parallel to the c-axis through half the atoms of *both* the adjacent layers and the other half lie along

[6] R. Vogel and G. Tamman, *Zeit. f. Phy. Chemie*, 1910, **69**, 598.

[7] Jacquelain, *Ann. d. Chim. et Phy.*, 1847, **20**, 468.

[8] A. R. v. Schrötter, *Sitzungsber. Akad. Wiss. (Wien)*, 1871, **63**, 465.

[9] H. Moisson, *The Electric Furnace; Comptes Rendus*, 1893, **117**, 423.

[10] G. Rose, *Monatsber. Berliner Akad.*, 1872, p. 685.

[11] Hon. C. A. Parsons and A. A. C. Swinton, *Proc. Roy. Soc. (A)*, 1907, **80**, 184.

[12] W. H. Bragg and W. L. Bragg, *Proc. Roy. Soc. (A)*, 1913, **89**, 27.

[13] W. Ehrenberg, *Zeit. f. Kristall.*, 1926, **63**, 320.

[14] J. D. Bernal, *Proc. Roy. Soc. (A)*, 1924, **106**, 749.

[15] O. Hassel and H. Mark, *Zeit. f. Phy.*, 1924, **25**, 317.

the lines parallel to the c-axis through the centres of the hexagons of the adjacent layers, the C-C distance being 1·42 Å and the distance between the layers being 3·41 Å. Knowing the above structures of diamond and graphite, one can now raise a very natural and important question as to how we can derive the graphite structure from the diamond structure. In the following we have proposed a solution for the above question and have calculated the temperature at which diamond converts to graphite, which agrees well with the experimental determinations of the said temperature.

In Part I,[16] it has been pointed out by the author that the vibration of the two cubic face-centred lattices of diamond is Raman-active but infra-red inactive and that it has the frequency 1332 cm.[1] We now seek the origin for the conversion of diamond to graphite in the above vibration which is triply degenerate. When the temperature of the crystal is raised or when its internal energy is increased, the amplitude of the vibration increases. At a definite increase of the amplitude, we will show in the following that the instability of diamond sets in. We will first give the purely geometrical aspect of the problem and then present its physical aspect in which we have followed a method for calculating the temperature at which the lattice vibration is of sufficient amplitude to favour the diamond-graphite transformation.

3. *The Geometrical Aspect of the Transformation.*

In the following we will show that the diamond structure can be transformed to a graphite-like structure by the following elementary operations:--

> (a) A definite displacement of the two cubic face-centred lattices of diamond relative to one another along a particular direction (Figs. 1 and 2).
>
> (b) A definite homogeneous dilatation of the whole crystal along the same direction (Fig. 3).
>
> (c) A definite homogeneous gliding of the planes obtained by the above two transformations perpendicular to the same direction (Fig. 4.)

(a) If we displace the two cubic face-centred lattices of diamond relative to one another along a diagonal of the unit cubic cell by 1/12 the length of the diagonal or increase the length of the C-C bonds parallel to the diagonal by a third of it, we get a structure which resembles apparently the graphite structure and which we call it as the α-pseudo-graphite structure. The following are its properties:--

---

[16] N. S. Nagendra Nath, *Proc. Ind. Akad. Sci. (.1)*, 1934, **1**, 333.
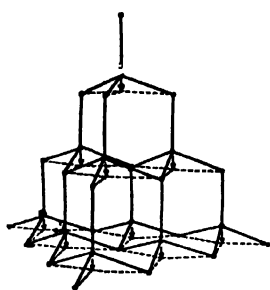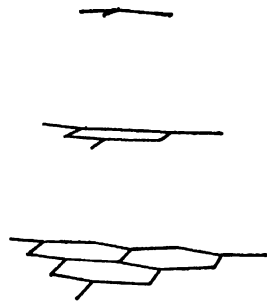
Fig. 1.   Fig. 2.   Fig. 3.

Fig. 1—Diamond structure→ α-pseudo-graphite structure.
Fig. 2—α-pseudo-graphite structure.
Fig. 3 —β-pseudo-graphite structure.
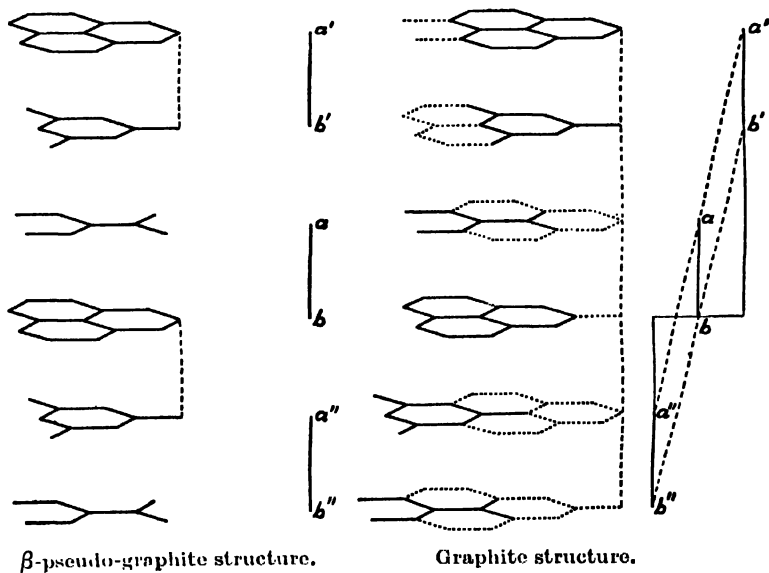
β-pseudo-graphite structure.   Graphite structure.

Fig. 4.

(1) The α-pseudo-graphite structure is similar to the graphite structure in that it is made up of equidistant parallel plane layers of carbon atoms, each layer being a hexagonal net. But half the atoms in a layer A are directly along the normals to the planes through half the atoms of only one

of the adjacent layers B and the same set of half the atoms in A are directly along the normals to the planes through the centres of the hexagons of the remaining adjacent layer C ; the remaining set of half the atoms in the layer A will be directly along the normals to the planes through half the atoms in the layer C and the same atoms in A will be directly along the normals through the centres of the hexagons in B.

(2) Assuming the C-C distance in diamond to be $1 \cdot 54$ Å, we can easily calculate the C-C distance in a plane of the α-pseudo-graphite structure. It is $1 \cdot 54$ sin [$\cos^{-1} (-\frac{1}{3})$] Å $= 1 \cdot 54 \times 2 \sqrt{\frac{2}{3}}$ Å $= 1 \cdot 45$ Å. This agrees pretty closely with $1 \cdot 42$ Å in graphite determined by X-ray analysis.

(3) The distance between the plane layers in the α-pseudo-graphite structure is $(1 \cdot 54 + 1 \cdot 54/3)$ Å or $2 \cdot 05$ Å while it is $3 \cdot 11$ Å in graphite.

(*b*) This transformation is only a simple homogeneous dilatation of the α-pseudo-graphite structure along a normal to its planes by the ratio $3 \cdot 11/2 \cdot 05$ or $1 \cdot 66$. We will call the transformed structure after the above operation as the β-pseudo-graphite structure which is practically identical with the graphite structure in the C–C distance, the distance between the layers and the structure of atoms in each layer except the symmetry of the planes taken as a whole. For example, every plane is a plane of reflection in the case of the graphite structure while it is not so in the case of the α- or β-pseudo-graphite structures. The next process to be described consists in transforming the β-pseudo-graphite structure into the graphite structure.

(*c*) The successive plane layers in the β-graphite structure may be symbolically represented by

$$\ldots \quad A \quad B \quad C \quad A \quad B \quad C \quad \ldots$$

and those in graphite may be represented by

$$\ldots \quad A \quad B \quad A \quad B \quad A \quad B \quad \ldots$$

where all the atoms in any of the planes A, B, C cannot be superposed on the atoms of any one of the remaining by the motion of the plane along a normal to the parallel planes. This is obvious in virtue of the property (1) in (*a*).

The required transformation of the β-graphite structure to the graphite structure is accomplished by considering a particular kind of gliding motion of the planes of the β-graphite structure. It consists in moving all the planes along a C–C direction lying in the planes with two adjacent planes as a rigid unit such that an unit suffers relative displacements with its adjacent units, which are equal in magnitude to the C–C distance but are opposite in sign. A clear pictorial representation of the transformation is given in the preceding figures.

4. The Physical Aspect of the Transformation.

It is clear from the above that there exists a simple continuous process by which we can derive the graphite structure from that of diamond. We shall now discuss its bearing on the physical aspect of the transformation.

We know that both diamond and graphite are normally stable substances. The stability of a crystal implies that the energy of the normal configuration is a minimum for all infinitesimal displacements of the lattice. With this idea in view, let us now discuss the course of the energy curve during the diamond-graphite transformation. While discussing it, *we ignore the forces between the layers in graphite as they are, we hope, exceedingly feeble compared to the valence forces in the planes.* As the process starts from diamond, the energy of the lattice increases in accordance with the fact that the energy of normal diamond is minimum for all infinitesimal displacements of the lattice. Similarly, starting from graphite to diamond the energy of the lattice also increases. As the process under consideration is a continuous one, the above means the existence of at least one stage at which the energy of the lattice is maximum, *i.e.*, a stage at which the energy of the lattice no longer increases either from the diamond side or the graphite side. But there cannot be more than one such stage or else it would imply the existence of minima of energy or of stable configurations in between the maxima distinct from those of diamond or graphite which seems to be highly physically improbable. In other words, the above considerations mean that there exists a single finite potential barrier between the diamond and the graphite states. We will call the stage of the lattice having the energy of the peak of the barrier as the α-stage.

If we now increase the thermal energy of diamond which is exhibited as the vibrational energy of its atoms, to such an extent that it will have the energy of the α-stage, the immediate instability of diamond will set in. Since diamond requires a definite temperature to possess a definite heat content from the thermal point of view, we can calculate the temperature at which diamond becomes unstable if we know the energy of the lattice at the α-stage.

The next question will be as to how to plot the energy curve during the process of transformation quantitatively. It appears to be exceedingly difficult to give even a fairly reasonable answer to this question. But we make here an approximation which will not be perhaps far from the truth. We know from molecular spectra that the course of the potential energy curve of a diatomic molecule is given for a fair approximation by a Morse function. In the case of diamond, we will assume a Morse function characterised by the constants D and *a* for all the chemical bonds in it. As the

Morse function is generally valid for fairly large variations of the internuclear distance of a diatomic molecule, we can assume the Morse function of the C-C bonds in diamond to be valid so long as diamond is stable. That is, the Morse function of the C-C bonds in diamond may be regarded as valid till the α-stage. To get an approximate function for the energy curve between the α-stage and the graphite state, we think of the transformation of the graphite structure to that of diamond. Regarding the C-C bonds in the layers of graphite to be all identical in character so far as our calculations are concerned here, we assume a Morse function characterised by the constants D' and a' for all the C-C bonds in graphite and also assume that it is valid between the graphite state and the α-stage. One may say that our approximation is weak in this case for we have ignored the forces between the layers. It is no doubt a defect, but it is one which cannot be remedied immediately and justifiably. However, we believe a part of the defect is compensated for in our assumption that the C-C bonds in the planes of the graphite structure will have the same character till the α-stage instead of the fact that there is a gradual weakening of the C-C bonds in the planes and the gradual appearance of the C-C bonds perpendicular to the planes.

The dissociation energy D of a normal C-C bond[17] is 72·6 K. cal./gm. mol. We know from the data on the Raman spectra of organic compounds that the force constant of the C-C bond is in the neighbourhood of about 4×10^5 dyn./cm., the expression for the force constant from the Morse formula being $2Da^2/N$. From this, it follows that a is $\backsim 2 \times 10^8$. Now we can regard the dissociation energy of a C-C bond in graphite to be $1\frac{1}{3}$ times that in diamond for it is observed by Roth[18] and his collaborators that the heats of combustion of diamond and graphite are practically identical. So D' is 96·8 K. cal./gm. mol. We now assume that the force constant of a C-C bond in graphite is about $1\frac{1}{3}$ times that in diamond. To this assumption, we attach no physical significance whatsoever; we are only aiming at an approximate value of the force constant of a C-C bond in graphite. With this assumption $a' \backsim 2 \times 10^8$.

Let r' be the internuclear distance of the C-C bonds parallel to the line of displacement and r be the internuclear distance of the other C-C bonds

[17] K. Fajans, *Ber. der Deut. Chem. Ges.*, 1920, **53**, 643; 1922, **55**, 2826.

Prof. K. Fajans gives the values 75 K.cal./gm. mol. and 100 K. cal./gm. mol. for D and D' respectively. The value 72·6 K. cal./gm. mol. for D has been taken from the work of Prof. E. Hückel. This difference does not affect our calculations in this paper to any appreciable extent.

[18] W. A. Roth and H. Wallasch, *Ber. der Deut. Chem. Ges.*, 1913, **46** (1), 896.

W. A. Roth, *Zeil. f. Electrochem.*, 1915, **21**, 3.

W. A. Roth and G. Naeser, *Zeit. f. Electrochem.*, 1925, **31**, 461.

equally inclined to the line of displacement.   As we proceed from the diamond state to the a-stage r' is a definite function of r as there is only one co-ordinate during that interval.

If E_a corresponds to the energy gained by a gram-atom of the diamond lattice when it comes to the a-stage,

$$E_a = \frac{D}{2}\left[\left\{1 - e^{2(r'_a - 1\cdot54)}\right\}^2 + 3\left\{1 - e^{-2(r_a - 1\cdot54)}\right\}^2 \right.$$

$$= \frac{3D'}{2}\left\{1 - e^{-2(r_a - 1\cdot42)}\right\}^2 \tag{1}$$

where $2D$ and $3D'/2$ are the dissociation energies of gram-atoms of diamond and graphite respectively and r's are given in Ångstrom units and their values at the a-stage are denoted by the subscript a.   Solving the equation (1), we find

$$r'_a = 1\cdot68\ \text{Å}\quad \text{and}\quad r_a = 1\cdot50\ \text{Å}.$$

Hence the a-stage in the process of the transformation occurs when the two cubic face-centred lattices are displaced apart along a diagonal of the unit cell by $(1\cdot68 - 1\cdot54)$ Å or $0\cdot14$ Å.   We also find

$$E_a = 2920\ \text{cals./gm. atom.}$$

The potential energy curve of the transformation is drawn in Fig. 5.
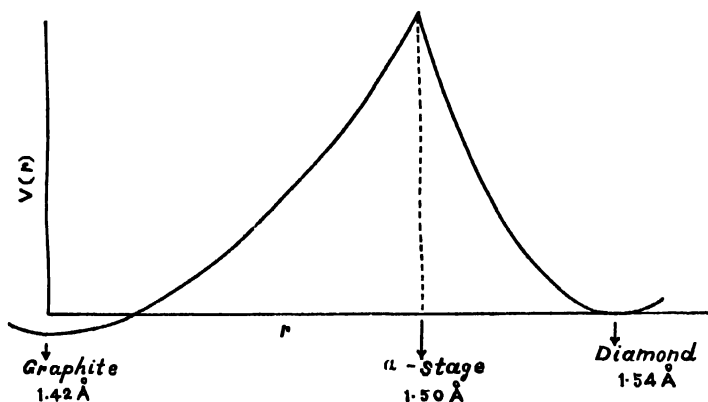
FIG. 5—The potential energy curve between the diamond and the graphite states as a function of r.   The initial energy of the diamond lattice is taken as zero.

The above calculations mean that the diamond lattice will only be stable if the change in the C–C distance parallel to the line of displacement does not exceed $0\cdot14$ Å.   So, if we increase the thermal energy of diamond which is exhibited as the vibrational energy of its atoms to such an extent that the lattice vibration will have an amplitude of $0\cdot14$ Å, the instability

of diamond will set in. Since the lattice vibration is triply degenerate, the maximum heat content of diamond without its being unstable is $2920 \times 3 = 8760$ calories/gm. atom. We make now a calculation of the temperature at which the instability of diamond will set in and at which diamond will have the maximum heat content.

Using the Einstein formula for the heat content, we get

$$\frac{3\,R\,\Theta}{\exp(\Theta/T) - 1} = 8760 \qquad\qquad (2)$$

where $\Theta = h\nu/k$, where the frequency ν corresponds to the characteristic frequency expressed in absolute units. If we assume that the characteristic Einstein frequency of diamond has a magnitude equal to the Raman frequency 1332 cm.$^{-1}$ (in wave numbers), we get $\Theta = 1907$. Solving (2), we get

$$T \approx 2290 \text{ A.}$$
$$\approx 2000^\circ \text{ C.}$$

The temperature T may also be calculated by the use of the Nernst-Lindemann formula for the heat content of a crystal, which gives satisfactory results for the specific heat of diamond at higher temperatures. According to it

$$\frac{3}{2} R \left[\frac{\Theta}{\exp(\Theta/T) - 1} + \frac{\Theta/2}{\exp(\Theta/2T) - 1} \right] = 8760 \qquad (3)$$

where the value for Θ is 1910 as given by Nernst and Lindemann[19] themselves. Solving the equation (3), we get

$$T \approx 2110 \text{ A.}$$
$$\approx 1850^\circ \text{ C.}$$

These calculations of the temperature at which diamond converts to graphite stand in good agreement with the experimental determinations which lie between 1650° C. and 1885° C., the temperature of rapid transformation being about 1750° C. according to M. de Kay Thompson and P. K. Frölich and Emerich Szarvasy and Béla Lányi.

The author is highly thankful to his professor Sir C. V. Raman for his great and helpful interest in this work.

5. Summary.

The transformation of the diamond structure to the graphite structure is explained in terms of three elementary operations, one of which is a definite displacement of the two cubic face-centred lattices of diamond relative to one another. It is shown here that for a certain displacement of the

[19] W. Nernst and F. A. Lindemann, *Ber. Berliner .Ikad.*, 1911, **1**, 494; *Zeit. f. Electrochem.*, 1911, **17**, 817.

component lattices, diamond attains maximum energy of its configuration
and becomes unstable. The temperature at which diamond becomes un-
stable and transforms to graphite is calculated and is shown to be in good
agreement with the experimental determinations.

The ideas followed up here seem to be not very special to the diamond-
graphite transformation itself but can have extensions for similar types of
transformation of other substances.

Note added in proof.—While this paper was in the Press, two papers by
W. Lasareff have appeared (*Jour. Phys. Chem.*, 1935, **39,** 913; *Physica*,
1935, **2,** 737) in which he has calculated the dissociation energy of a C–C
bond in diamond. The value given in *Physica* is $132 \pm 3 \cdot 5$ K. cals./mol. for
D, departing greatly from the values known till now which range between
$70 - 80$ K. cals. Lasareff has argued that a carbon atom in diamond is in
the (^5S) state while a carbon atom in the gaseous state is in the (^3P) state
and that the difference between the energies of these two states has to be
considered when calculating the dissociation energy of a C–C bond in
diamond by the help of the data regarding the sublimation heat. However,
it should be noted that this great change in the dissociation energy of a
C–C bond does not affect our calculations of E_a, as Da^2 the coefficient of the
quadratic term r^2 does not vary for it is equal to $NK/2$ where N is the
Avogadro number and K is the force constant of the C–C bond whose
value has been assumed to be $\approx 4 \times 10^5$ dyn./cm. Thus a change in D with
the corresponding change in a affects only higher order terms. Thus our
results should not be affected to a great extent by a change in D. We
have made calculations of a, a', r_a, r_a' and E_a assuming Lasareff's value
for D $(D' = 4D/3 = 176$ K. cal./mol.). The value of a is $1 \cdot 48 \times 10^8$ cm. [1] and
the values of r_a and r_a' are the same old values $1 \cdot 50 \text{Å}$ and $1 \cdot 68 \text{Å}$ respec-
tively. E_a would be 3040 cals./gm. atom. It follows from the above that
T is $\approx 2080°$C. according to the Einstein formula and that it is $\approx 1900°$C.
according to the Nernst-Lindemann formula. These values are again in
agreement with the experimental determinations.

REDETERMINATION OF THE DEPOLARISATION OF LIGHT SCATTERING IN GASES AND VAPOURS.

By R. Ananthakrishnan.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. Introduction.

IN a previous communication to these *Proceedings*,[1] the author has discussed at length the question of the dependence of the observed value of the depolarisation on the finite convergence of the incident beam, when, as is usual in dealing with gases and vapours of small depolarisation, a condensing lens is employed to concentrate the light on the scattering medium. In view of the conclusion arrived at theoretically, and confirmed experimentally that *the depolarisation of the transversely scattered light measured at the focus is definitely higher than the genuine value*, the magnitude of the deviation depending in a perfectly determinate way on the convergence of the incident beam, it appeared to be highly important to take up a systematic redetermination of the depolarisation in the case of a number of gases and vapours because of the uncertainty that attaches to the values reported by previous experimenters who have partially or totally ignored the correction for the convergence of the incident beam. The present paper contains the results of the experimental study of more than a dozen gases and vapours. The values of the depolarisation show in many cases not only a definite departure from those reported by previous workers, but present certain new and interesting features which will be discussed in the course of the paper.

2. Experimental Technique.

By far the greater portion of the experimental study of the depolarisation of gases and vapours is due to the pioneer work of Lord Rayleigh in England, of Cabannes and his co-workers in France, and of Prof. Raman and his students at Calcutta. A critical review of the experimental technique of the different workers is given in Cabannes' book.[2] While Lord Rayleigh and Prof. Raman have employed metallic crosses of large capacity painted black inside, with suitable diaphragms to protect against parasitic light, Cabannes and his co-workers have mostly used smaller crosses made of glass

[1] R. Ananthakrishnan, *Proc. Ind. Acad. Sci.*, A, 1935, **2**, 133.

[2] J. Cabannes, *La Diffusion Moleculaire de la Lumiere*, 1929.

A4   F

with no diaphragms inside, and painted black externally. Although each technique has its own advantages, experience has shown the present author that the best arrangement is one in which the advantages of both the above modes of experimentation are sought to be combined. Towards this end, a cross in pyrex glass of over a litre in capacity was made with the following approximate dimensions. The tube taken was 4 cms. in diameter, and three of the four arms were about 20 cms. in length. The fourth arm opposite the observation side was much longer and was drawn out and bent up in the shape of a horn to the end of which was attached a pyrex stopcock for admission of gas into the cross. Although the use of diaphragms may not be very important when the photographic method is adopted for depolarisation measurements, accurate visual photometry is practically impossible unless the utmost precautions are taken to avoid parasitic light from entering within the field of observation. Suitable diaphragms of oxidised brass were therefore put in inside the cross. These diaphragms were made out of short lengths of thin brass tube one of the ends being closed by a thin brass plate having a central hole of $\frac{1}{4}$ inch diameter. The surfaces of the diaphragms were rendered black by immersing them in an ammoniacal solution of copper carbonate. They were then rolled in thin asbestos paper and introduced into the arms of the cross. After fixing the positions of the diaphragms within the cross by a preliminary experiment, the open ends of the three arms were closed by fusing on flat pyrex plates (previously examined between crossed nicols for strain) in the way recommended by Martin.[3] The whole cross excepting the end windows was painted dull black externally. The complete eschewal of all traces of paint from within the cross ensured perfect freedom from contamination of the gases and vapours studied.

The cross was mounted on a stool provided with levelling screws and spirit level, and observations were made within a dark cabin into which sunlight was reflected by a single mirror Foucault heliostat. A Dallmeyer photographic lens of adjustable aperture, and focal length 1 foot fixed to the wall of the cabin served to concentrate the sunlight at the centre of the cross. The whole arrangement was such as to give an exceedingly nice background against which even the faintest tracks could be seen with but little difficulty.

All the gases were taken directly from commercial cylinders supplied by the Ohio Chemical and Manufacturing Co., and were guaranteed a high degree of purity. The scheme of the experimental arrangement is shown diagrammatically in the figure. The gases were admitted into the cross at atmospheric pressure through a tube of anhydrous P_2O_5 and a long plug

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[3] W. H. Martin and S. Lehrman, *Jour. Phys. Chem.*, 1922, **26**, 76.
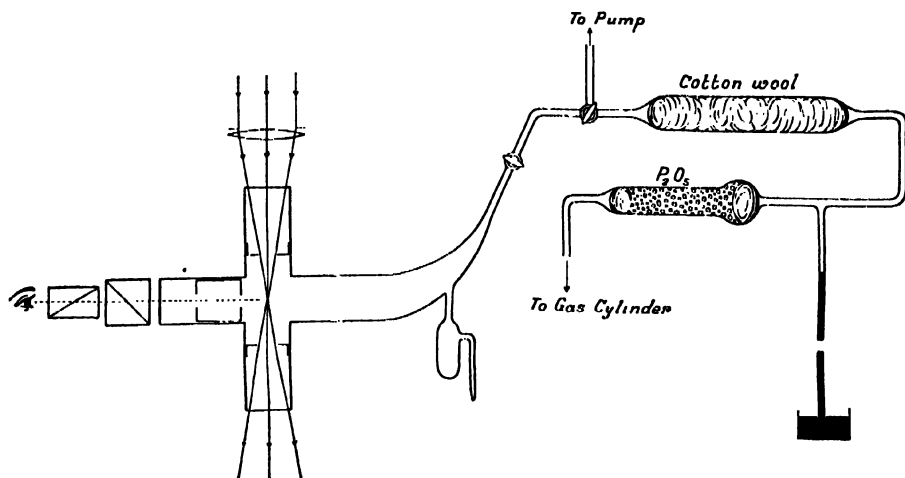
of compressed cotton wool after evacuating the whole system by means of a Cenco Hyvac Pump. Rubber connections were avoided as far as possible to minimise leaks, and special care was taken to see that the system was perfectly leak-tight before commencing the experiments. Estimates of depolarisation were made visually by the usual Cornu method.

In the case of CCl_4, the technique adopted was slightly different. Extra-pure CCl_4, guaranteed to be free from CS_2, was refluxed with freshly distilled mercury for several hours and finally distilled at constant boiling point. A small quantity of the liquid was introduced into the bulb attached to the longer arm of the cross by means of the side tube and the latter was then sealed off. The CCl_4 was frozen in liquid air, and the cross was exhausted to the highest vacuum. The stopcock was then closed, and the liquid air bath removed. As the CCl_4 rises up to the room temperature, the vapour pressure is quite appreciable to give a scattering of sufficient intensity to make accurate measurements.

3. *Adjustments and Sources of Error.*

The accurate estimation of small depolarisation values with which one is confronted in the case of the majority of gases and vapours necessitates the utmost precautions to safeguard against various sources of error which tend to vitiate the observations. Unpolarised light diffused by the background is highly detrimental in the case of visual photometry as has been already pointed out. The error arising from this source is got rid of by the use of suitable diaphragms, and by viewing the two images of the track seen through the double image prism against the same background. Horizontality of the axis of the incident beam is very important, and is secured

by setting the cross horizontal with the spirit level. Normality of the direc-
tion of observation to the axis of the incident track is easily secured when
the container is cross-shaped. The principal section of the double image
prism is set vertical by viewing an illuminated plumb line, and rotating the
prism about a horizontal axis till the two images are entirely superposed.

The effect of the finite convergence of the incident beam is to enhance
the value of the depolarisation, and the correction to be applied to the ob-
served value under this head is given by $\theta^2/2$, where θ is the semi-angle of
convergence. It may be remarked that the experimental results of Dr. I.
Ramakrishna Rao[1] and the later work of Parthasarathy[5] require correction
in this direction.

4. Results.

The table below gives the experimental results after being corrected
for the convergence of the incident beam. An aperture of F/5·6 was
employed in all cases and the correction required for the observed value
can be easily seen to be 0·4%.

Gas		% Purity	Depolarisation
Methane	CH_4	96·5	0·34%
Ethane	C_2H_6	90	0·50%
Propane	C_3H_8	99·9	0·66%
N. Butane	C_4H_{10}	99	0·85%
Isobutane	C_4H_{10}	99	0·46%
Cyclopropane	$\begin{matrix} H_2C \\ H_2C \triangle CH_2 \end{matrix}$	99·5	0·52%
Propylene	C_3H_6	99·5	2·91%
Methyl Chloride	CH_3Cl	99·5	1·95%
Ethyl Chloride	C_2H_5Cl	99·5	1·49%
Carbon Tetrachloride	CCl_4	..	0·15%
Dimethyl Ether	$CH_3 \cdot O \cdot CH_3$	99·95	1·20%
Argon	A	86	0·42%
Hydrogen Sulphide	H_2S	99·73	0·30%
Nitrous Oxide	N_2O	98	12·95%

[4] I. R. Rao, *Ind. Jour. Phys.*, 1927, **2**, 61.

[5] S. Parthasarathy, *Ind. Jour. Phys.*, 1932, **7**, 139.

5. *Discussion of Results.*

As has been already remarked, the results of the author are, in many cases, entirely at variance with those of previous experimenters.

Methane.—We shall commence with methane, the leading member of the paraffin series. The first outstanding result of the investigation is that the genuine depolarisation factor of methane is considerably lower than what it has been till now assumed to be. While Cabannes gave the value 1·5% and Parthasarathy 1·12%, the actual value appears to be of the order of 0·3%. Considering the fact that the residual impurities in methane are most probably some of the higher hydrocarbons whose depolarisation factors are quite small, the observed depolarisation of methane seems to be quite genuine. Thus, for instance, if we assume that the 3·5% impurity is all ethane whose intensity of scattering is roughly thrice as great as that of methane, the observed depolarisation of methane will be affected only in the second place of decimals. It is not improbable that the source of this depolarisation is to be sought for in the existence of depolarised Raman scattering. The Raman Spectrum of methane shows an intense line at 2915 cm.$^{-1}$ corresponding to the total symmetric vibration of the molecule, which according to the observations of Bhagavantam[6] is depolarised to the extent of 8%, but possesses no rotational fine structure. On the other hand, the intense band at 3022 cm.$^{-1}$ is highly depolarised to the extent of 80%, and shows equispaced rotation lines on either side of it. Stuart[7] seems to be of opinion that this strongly depolarised vibration band would be responsible for a spurious depolarisation of the Rayleigh radiation to the extent of 0·1 to 0·5%, when one is working with white light as is usual in depolarisation measurements. Thus, in the present case where sunlight was employed, the photographic lens used for focussing the beam transmits the far violet end of the spectrum, say up to 3000 Å U. and the corresponding vibration scattering would fall well within the region of greatest visibility. If this view be correct, and a filter be employed which cuts off the entire violet and blue regions of the spectrum on the incident side, and another which cuts off the entire region beyond the green and yellow be employed on the observation side, we should expect the depolarisation of methane not to differ from zero. All these considerations lend strong support to the view that the Rayleigh scattering in the case of methane is completely polarised. This conclusion is in perfect accord with the observation of Bhagavantam,[8]

[6] S. Bhagavantam, *Nature*, 1932, **129**, 830.

[7] H. A. Stuart, *Molekulstruktur*, 1934, p. 192.

[8] S. Bhagavantam, *Nature*, 1932, **130**, 740.

later confirmed by Lewis and Houston,[9] that even prolonged exposures extending over a week fail to bring out any rotation wings for the Rayleigh line in the case of methane. The assumption of the anisotropy of the carbon atom thus appears to be definitely uncalled for to explain the depolarisation of methane. In this connection, a redetermination of the depolarisation factor of the total symmetric vibration line would be of great interest, since, as has been remarked by Stuart,[10] Bhagavantam's value appears to be too high.

Higher homologues.—The second important outcome of the experimental work is the fact that as we pass from methane to the higher homologues, the depolarisation shows a steady increase from 0·34% in the case of methane to 0·85% in the case of normal butane. Cabannes, however, arrived at the conclusion that the depolarisation does not change appreciably from one member to another, but the conclusion of the author is supported by the observations of Parthasarathy.[11] It thus appears that anisotropy is not wholly unconnected with the geometric form of the molecule. That this is so is again shown by the fact that while normal butane has a depolarisation of 0·85%, for isobutane the depolarisation drops down to 0·46%, in conformity with the greater symmetry of the molecule.

Other hydrocarbons.—Cyclopropane, whose depolarisation does not appear to have been measured by any of the previous workers, shows a very small value, which is not surprising in view of the known low depolarisation of Cyclohexane. Propylene shows a high depolarisation, as would be expected on account of the double bond.

Methyl and Ethyl chlorides.—Methyl chloride is definitely more anisotropic than ethyl chloride, contrary to the observations of Cabannes. The large increase in the value of the depolarisation which results from the substitution of one atom of hydrogen in methane by an atom of chlorine is very significant. While this observation is in qualitative agreement with the results of Parthasarathy, it is in total disagreement with those of Cabannes who finds very little change between the depolarisation factors of methane and methyl chloride.

Carbon tetrachloride.—The depolarisation of the tetrahedral molecules has remained for a long time a puzzling and unintelligible problem. For CCl_4, the depolarisation in the vapour state was estimated to be 0·77% by Cabannes, 0·5% by Ramakrishna Rao and 0·62% by Parthasarathy. Stuart[12] finds these values to be wholly incompatible with

[9] M. Lewis and W. V. Houston, *Phys. Rev.*, 1933, **44**, 903.
[10] H. A. Stuart, *loc. cit.*, p. 329.
[11] S. Parthasarathy, *loc. cit.*
[12] H. A. Stuart, *loc. cit.*, p. 193.

his measurements of the Kerr-constant of CCl_4 from which he concludes that the departure of the molecule from spherical symmetry is imperceptibly small, and would not be responsible for a depolarisation of the vapour exceeding 0·15%. The author's result stands in good agreement with this value. It should, however, be pointed out that the experimental value itself gives only an upper limit for the depolarisation, since in the actual measurements, all possible sources of error only tend to enhance the genuine value, and also because, here, one is well-nigh at the limit of accuracy in depolarisation measurements. As Stuart[13] has pointed out, the investigation of the fine structure of the Rayleigh line, as well as that of the total symmetric vibration Raman line in the case of CCl_4 vapour, would be of extreme interest in this connection.

It might on first consideration seem conceivable that a small anisotropy would be caused in the case of CCl_4 by the presence in one and the same molecule of chlorine isotopes of different masses (35 and 37). However, on account of the physical similarity of isotopes with nearly equal masses, the magnitude of this anisotropy would appear to be vanishingly small.

Argon.—The case of argon demands special explanation. In the first place, it will be noticed that the sample employed is very impure. The most probable impurity appears to be nitrogen and if it be assumed that the 14% impurity is all nitrogen, it follows from simple calculation that the depolarisation of argon is not sensibly different from zero, as the depolarisation of nitrogen is about 3%, and the relative scattering powers of argon and nitrogen are not very different.

The reality or otherwise of the depolarisation of the rare gases has been an unsettled issue. Experimentally, Cabannes appears to have found a small depolarisation of 0·5% for argon, krypton and xenon, while for neon the upper limit is given as 1%. The case of helium is again very uncertain, the upper limit for its depolarisation being given as 6·5% by Lord Rayleigh and as 3% by Parthasarathy. From the theoretical standpoint these values are difficult to comprehend. The ground state of the atoms of the rare gases is the ¹S state, and theory indicates that the scattered radiation corresponding to such terms should be linearly polarised. Placzek[14] has therefore expressed himself as very sceptical of the reality of these depolarisation values. The author's experimental results indicate that the finite depolarisations reported by earlier workers are not trustworthy. However, this conclusion is only tentative, and the author hopes, before long, to take up

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13 H. A. Stuart, *loc. cit.*, p. 194.

14 G. Placzek, *Quanten Mechanik der Materie und Strahlung*, Teil II, Leipzig, 1934, p. 259.

the purification of argon, and a direct experimental test of the anisotropy of this gas, as well as of other rare gases, particularly helium.

Hydrogen sulphide.---H_2S shows an extremely small depolarisation (0·3%) the reality of which however is quite definite. Of the previous workers, Ramanathan estimated the depolarisation as 1% and Parthasarathy as 0·93%. The depolarisations for other hydride molecules such as HCl, HBr, NH_3, etc., as estimated by Parthasarathy are also of the same order of magnitude.   In view of the author's low value for H_2S, it would seem that the other values would also be similarly low.   These very low values are not difficult to understand, since, as Stuart[15] remarks, " die Hydrid-moleküle HCl, H_2O, H_2S und NH_3, die wir als Pseudoedelgase mit mehreren unpolarisierbaren H-Kernen in einer gemeinsamen Elektronenwolke auffassen können, trotzdem sie elektrisch unsymmetrisch sind, d.h. ein elektrisches Moment besitzen, einen sehr hohen optischen Symmetriegrad haben".

In conclusion, the author wishes to record his grateful indebtedness to Professor Sir C. V. Raman for valuable guidance and suggestions in the course of the present work.

6. *Summary.*

It is pointed out that the existing depolarisation data of gases and vapours are gravely defective, and a redetermination of the values has been made with improved experimental technique. The results obtained are strikingly different from those of previous workers, and afford at the same time a natural explanation of many of the existing anomalies.   The genuine depolarisation of methane appears to be only of the order of 0·3% which is in all probability to be attributed to the highly depolarised vibration Raman lines.   The depolarisation shows a steady increase as one goes to the higher members of the homologous series, but the values in all cases are much smaller than they were hitherto assumed to be. Cyclopropane shows a very small depolarisation in conformity with the known small depolarisation of cyclo-hexane, while propylene shows a high value as would be expected from the presence of the double bond in it.   Methyl chloride is more anisotropic than ethyl chloride, and the depolarisation factor of the former is much higher than that of methane. Carbon tetrachloride shows an extremely small depolarisation of 0·15% which is discussed in detail.   It is tentatively concluded that the depolarisation of argon is nil.   The depolarisation of H_2S is only 0·3%.   An explanation is offered for the low depolarisation of the hydride molecules in general.

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15 H. A. Stuart, *loc. cit.*, p. 190.

INFLUENCE OF THE FORMATION OF HYDRATES ON THE DIAMAGNETISM OF CHEMICAL COMPOUNDS.

BY P. S. VARADACHARI, M.A., M.Sc.,

Annamalai University, Annamalainagar, South India.

1. Introduction.

IN a previous paper[1] (hereafter referred to as Part I) a study was made of the diamagnetic susceptibilities of liquid mixtures from the point of view of additive law. It is shown that magnetic measurements are not sensitive to interaction effects, whether between like or unlike molecules, carrying high dipole moments. Break-up of association by heating or by the addition of foreign molecules does not produce any change in the value of the mass susceptibility. In this paper, a study has been made of the influence of chemical action on diamagnetism, particularly when hydration is concerned.

Recently Cabrera and Fahlenbrach[2] working with aqueous solutions of potassium iodide observed a small but definite change in the susceptibility value which they attribute to hydration. They suggest that this change is due to the deformation of the ion brought about by hydration. It is with the object of verifying whether such small changes could be observed in magnetic measurements that a study of the decahydrate of sodium sulphate (Na_2SO_4, $10H_2O$), both in solution and in the solid state was undertaken. This salt commends itself as specially suited for this purpose since, as is well known, it decomposes into anhydrous Na_2SO_4 on heating to temperatures above 33°C.

A study of sulphuric acid-water mixtures has also been carried out since several hydrates of the acid are known. The fact that this acid (which is an electrolyte) gives a change in susceptibility on forming a compound gives additional interest to this study. Farquharson[3] has studied this mixture with the Curie-Cheneveau balance. His concentration-susceptibility curve deviates considerably from the additive law and abounds in maxima and minima.

Another mixture which has been studied is acetic acid-water. This is one of great importance since evidence regarding the formation of a compound at equimolecular concentrations is quite definite both from Raman effect data and from viscosity measurements. Besides, Sibaiya and

[1] *Proc. Ind. Acad. Sci.,* 1934, **1**, 77.

[2] *Zeits. f. Phys.,* 1934, **89**, 166.

[3] *Phil. Mag.,* 1931, **12**, 283.

Venkataramiah[4] have studied the magnetic properties of the liquid mixture and showed that the diamagnetic susceptibility decreased considerably (by about 12 per cent.) at equimolecular concentrations. Hence a study of this mixture has been made at different temperatures to test whether the compound formation has any effect on susceptibility. If so, a progressive variation must be noted when an equimolecular mixture is heated. Such deviations have not been observed in the present investigation.

TABLE I.
Sulphuric acid-water.

Sample I		Sample II		Sample III	
Percentage of acid by weight	χ	Percentage of acid by weight	χ	Percentage of acid by weight	χ
0·0	0·720	0·0	0·720	0·0	0·720
7·9	0·686	53·5	0·535	5·1	0·700
21·5	0·628	54·1	0·526	12·3	0·673
40·7	0·565	59·5	0·510	17·8	0·650
46·5	0·541	63·1	0·493	29·8	0·608
49·1	0·540	76·9	0·453	32·5	0·598
53·2	0·529	79·4	0·445	44·5	0·556
65·2	0·482	82·9	0·431	58·8	0·511
71·2	0·469	86·5	0·428	63·1	0·508
82·5	0·432	87·3	0·425	66·0	0·483
97·0	0·403	87·6	0·426	69·4	0·477
		90·5	0·423	69·8	0·474
		93·3	0·416	76·2	0·451
		97·0	0·402	94·5	0·412
				97·4	0·403

2. *Experiment.*

Two methods of determining the susceptibilities were employed. (*a*) Quincke's method, the details of which are given in Part I and (*b*) Curie method[5] in the special case of sodium sulphate.

4 *Ind. Jour. Phys.*, 1932, **7**, 393.
5 Full details of the method are given in *Proc. Ind. Acad. Sci.*, 1934, **1**, 123.

3. Results.

(a) *Sulphuric acid-water.*—The sulphuric acid used was analytically pure and was taken from freshly opened bottles. Three samples were used. The densities of the pure acid and the mixtures were determined by weighing a clean glass piece suspended by a fine platinum wire, in air, in water and in the liquid. From a knowledge of the density, the concentration of the acid could be found from the tables.

Careful experiments were conducted to test for the presence of iron or other ferromagnetic impurities in the acid. A direct chemical test failed to show the presence of iron. Further the values of the magnetic susceptibility of some mixtures were determined at different field strengths. Results for any given mixture gave a constant value showing the absence of any ferromagnetic impurities.

The mean values obtained for the three samples of sulphuric acid at different concentrations are given in Table I.

Fig. 1 shows the curve between composition and susceptibility. It
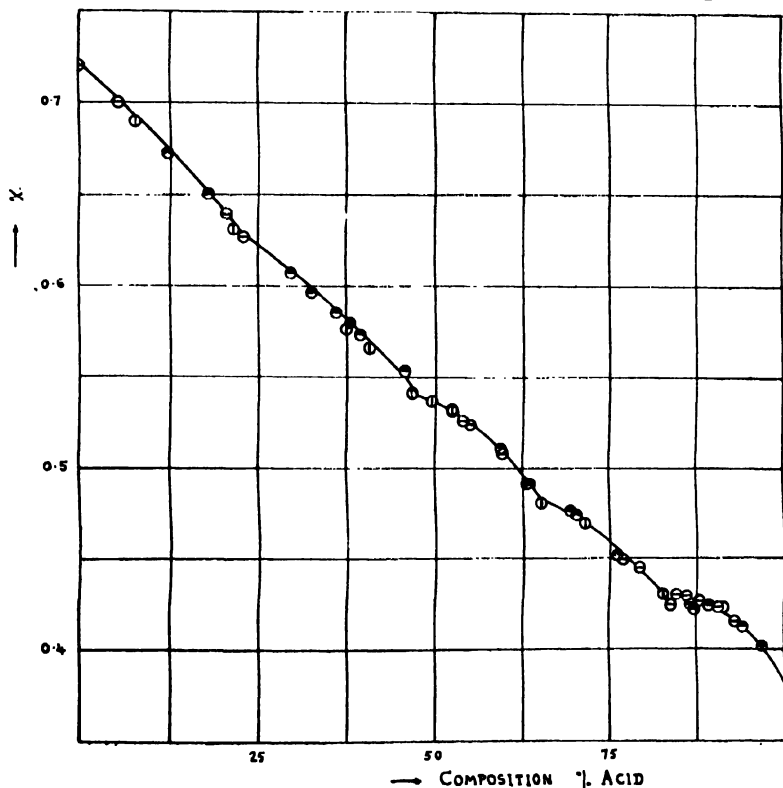
FIG. 1.

is observed that there is considerable departure from the additive law. Singular points in the composition-susceptibility curve may be taken as an indication of an interaction in the system. To find out these singular points better, the deviation from the additive law of mixtures has been plotted against the corresponding concentration. This has been done in Fig. 2. It is seen that the definite formation of hydrates is indicated in the figure, these being $2H_2SO_4, H_2O$; H_2SO_4, H_2O ; $H_2SO_4, 3H_2O$; $H_2SO_4, 6H_2O$ and $H_2SO_4, 18H_2O$. One point deserves special mention. The susceptibility value corresponding to $2H_2SO_4, H_2O$ is greater than the additive value while the values for the other hydrates are smaller than the corresponding additive values. This leads to the definite conclusion that the type of interaction in the case of $2H_2SO_4, H_2O$ is different from that of the other hydrates.
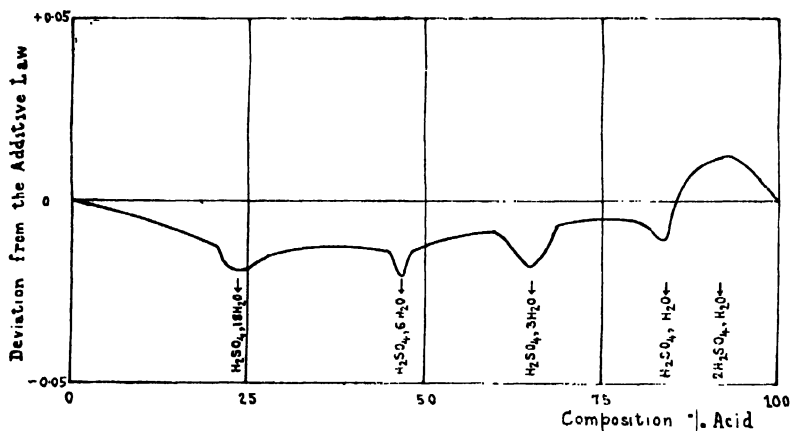
Fig. 2.

It is interesting to compare these results with those of Farquharson. His curve shows a flat maximum at 86-89 per cent. which he suggests as due to H_2SO_4, H_2O. He obtains also a break at the composition corresponding to $H_2SO_4, 3H_2O$. Between 0 and 50 per cent. of the acid, he obtains several minor maxima and minima for which he does not offer any explanation. He identifies the decided maximum at 15·1 per cent. as the state in which all the ions are free and obtains a value of 39·0 for the susceptibility of the SO_4 ion. In the formation of the hydrates H_2SO_4, H_2O and $H_2SO_4, 3H_2O$, Farquharson's results and those of the present investigation agree. Our results show clearly by the aid of Fig. 2 that singular points exist at compositions corresponding to a few more hydrates.

Attention may be drawn to the evidence available from other properties regarding the formation of hydrates.[6] The freezing point curves are usually examined to detect the presence of hydrates. It should however be pointed out that while they can be depended upon for solid hydrates separating from solutions, nothing can be inferred regarding the presence of these hydrates in solution. In fact it has been most conclusively shown that even the inert gases can separate out as hydrates on solidification at very low temperatures. This evidence clearly points to the fact that the affinity of the water molecules in the hydrates in the solid state may not be entirely chemical. On the other hand the existence of hydrates in solution points to a more fundamental link of the nature of a chemical bond between the water molecules and the substances under consideration. It is therefore necessary to look in other directions for the identification of the presence of hydrates in solutions.

Deviations from the additive law of mixtures have been plotted against the corresponding concentrations to find singular points by Morgan and Davis. They obtained evidence for the existence of the following hydrates :— H_2SO_4, H_2O by the specific gravity, viscosity and refractive index curves ; H_2SO_4, $2H_2O$ by the refractive index curve, H_2SO_4, $3H_2O$ by the surface tension curve and H_2SO_4, $12 H_2O$ by the conductivity curve. There is also evidence for the existence of H_2SO_4, H_2O from Graham's investigations on capillary transpiration, and from Thompson's thermochemical observations. Pfaundler and Schnegg discovered the existence of H_2SO_4, H_2O and H_2SO_4, $2H_2O$ from their cryscopic observations.

Bouty found in his electrical conductivity results a minimum for $2H_2SO_4$, H_2O and a maximum for H_2SO_4, H_2O. This is strikingly similar to the susceptibility results obtained in this investigation.

It is seen that hydrates of sulphuric acid having one, two, three, six and twelve molecules of water are all indicated by the different physical properties. The mono-hydrate is indicated by almost all the properties while the hydrate H_2SO_4, $4H_2O$ which crystallises out as a solid, is conspicuous by its absence in solution.

The magnetic observations corroborate the existence of the hydrates, $2H_2SO_4$, H_2O ; H_2SO_4, H_2O ; H_2SO_4, $3H_2O$; H_2SO_4, $6H_2O$ and H_2SO_4, $18H_2O$. The hydrate H_2SO_4, $2H_2O$ seems to be absent in the solution, an observation also made by Farquharson. One of two alternatives is possible. The di-hydrate may not be present in the solution under the conditions of the

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[6] The information contained in this and the following paragraphs has been taken from Mellor's *Dictionary of Applied Chemistry*, Vol. **10**, p. 353 ff.

experiments or the presence of the dihydrate may not give a break in the susceptibility-concentration curve. This problem needs further investigation.

Sulphuric acid-water mixtures have been studied from the point of view of the Raman Effect by Ramakrishna Rao,[7] Nisi[8] and Woodward.[9] The results show that the Raman frequencies 416, 562 and 1171 are affected considerably by the nature of the combination. The magnetic results outlined above, support the conclusions of Nisi that there are changes of frequency due to the formation of hydrates on dilution of the concentrated acid.

(b) *Acetic acid-water.*—Since the density difference between acetic acid and water is small, the method followed in the case of sulphuric acid-water mixtures to determine the composition of any mixture could not be adopted in this instance. Weighed quantities of the acid and water were mixed together and poured into the tube. On exhaustion and sealing, the loss of weight of the mixture by evaporation was only ½ per cent. of the total weight of the mixture. The composition of the liquid inside the tube was taken to be therefore the same as the value before exhaustion, the error in this determination being obviously less than ½ per cent.

TABLE II.

Acetic acid-water.

(Temperature 29° C.)

Acetic Acid %	Depression divisions on scale	χ
0	641·5	0·720
21·4	607·5	0·681
40·7	573·0	0·642
63·1	540·5	0·606
75·0	521·5	0·582
75·4	517·5	0·580
81·8	510·5	0·572
88·3	501·5	0·562
100·0	480·0	0·538

[7] *Ind. Jour. Phys.*, 1933, **8**, 123.
[8] *Jap. Jour. Phys.*, 1929, **5**, 119.
[9] *Phys. Zeits.*, 1931, **32**, 212; *Proc. Roy. Soc.*, 1934, **114**, 118.

The results for the susceptibility of acetic acid-water mixtures at different concentrations are given in Table II.

Table III gives the effect of temperature on the susceptibility of pure acetic acid and water.

TABLE III.

Acetic Acid			Water		
Temperature °C.	χ	χ_t/χ_{30}	Temperature °C.	χ	χ_t/χ_{30}
28	0·538	1·000	28	0·7200	1·0000
29	0·537	0·998	36	0·7202	1·0003
38	0·538	1·000	40	0·7206	1·0008
45	0·538	1·000	45	0·7211	1·0015
53	0·537	0·998	55	0·7216	1·0022
57	0·537	0·998			

The effect of temperature on the susceptibility of the mixtures is shown in Table IV.

TABLE IV.

Acetic Acid 88·3 %		Acetic Acid 75 %		Acetic Acid 21·4 %	
Tempera-ture °C.	χ	Tempera-ture °C.	χ	Tempera-ture °C.	χ
28	0·562	29	0·582	28	0·681
39	0·562	37	0·584	40	0·681
41	0·563	43	0·584	46	0·681
47	0·562	48	0·583	50	0·681
49	0·562	57	0·583		
50	0·562				
56	0·562				

It is seen from Table III that acetic acid shows no variation of susceptibility with temperature in spite of the fact that it has a high dipole moment and is highly associated at ordinary temperatures. The break-up of association in the case of acetic acid as in that of nitrobenzene produces no

change of susceptibility. The results accord well with those of Cabrera and Fahlenbrach.[10] In the case of water, on the other hand, a small definite increase is noted, the coefficient being nearly equal to the values obtained by Auer[11], Cabrera and Fahlenbrach[12] and Boeker[13].

In Fig. 3 is shown a graph between the percentage of acetic acid in the mixture and susceptibility. It is seen that the points plot smoothly on a straight line. No deviations such as were observed by Sibaiya and Venkataramiah are present in the graph.
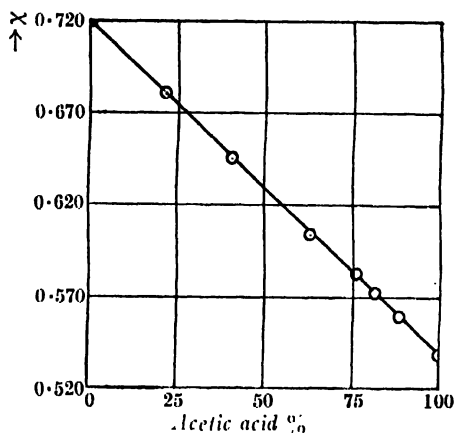
FIG. 3.

This mixture has special interest because of the fact that a definite compound is formed at the particular composition where the coefficient of viscosity is maximum. It is interesting to note that the position of the maximum in the composition-viscosity curve is independent of temperature showing the existence of a definite compound at that concentration.[14] The density curve also shows a maximum at the same concentration. Krishnamurthi[15] has investigated the mixture from the point of view of the Raman effect; his results show that in addition to 1667 cm.$^{-1}$ line characteristic of the acid, a new line at 1707 cm.$^{-1}$ appears at about 95% which increases in intensity with further dilution till it is the only line present at about 75% and lower concentrations of the acid. He further suggests from the intensity variations that the covalent linkage is strengthened in this case. However

[10] Zeits. f. Phys., 1934, 89, 682.
[11] Ann. der Phys., 1933, 18, 593.
[12] Zeits. f. Phys., 1933, 82, 759.
[13] Phys. Rev., 1934, 46, 907.
[14] Jour. Chem. Soc., 1909, 95, 1556.
[15] Nature, 1931, 128, 639.

no deviations from the additive law are caused by the compound formation in magnetic susceptibility measurements.

Increase of temperature produces no change in susceptibility. If any change of susceptibility is brought about by the compound formation, such changes must show alterations with temperature and departures from the additive law ought to occur at higher temperatures. The absence of such an effect proves that no measurable change has been caused by the formation of the compound and any change that takes place is too feeble to affect the electronic system considerably.

(c) *Sodium sulphate-water.*—This is a case of a strong electrolyte dissolved in water. It is wellknown that sodium sulphate is present in solution as the decahydrate below 33° C. and that it decomposes at higher temperatures. The reaction can be represented thus :—

$$Na_2SO_4, 10H_2O \overset{33°C.}{\rightleftharpoons} Na_2SO_4 + 10H_2O$$
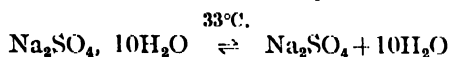
The forward reaction is almost instantaneous while the backward reaction takes some time to proceed, so that it is possible to have the anhydrous sodium sulphate below 33° C. for a short time. The solubility curve brings out this point quite clearly since the solubility of the hydrate is entirely different from that of the anhydrous salt.

These facts have been utilised to study the magnetic behaviour of the hydrated salt both in solution and the solid state. Ray-Chaudhuri[16] has determined the magnetic susceptibility of a number of salts when they are hydrated and anhydrous and finds that in a large number of cases, a marked deviation is observed between the calculated additive value of the hydrate and the observed value. He concludes from the investigation that the deviation from the additive law is more prominent in those cases where the heat of formation is large. The fact that the hydrated and the dehydrated salts were prepared separately for the magnetic investigations makes a quantitative study a little doubtful. An obviously better method would be to prepare the hydrated salt and determine the magnetic susceptibility before and after the dehydration, the water remaining in the combined state in one case and free in the other so that the effect of binding alone will be recorded by the change in the magnetic susceptibility value.

This method is not always easy to realise. But it happens that Na_2SO_4 lends itself easily to such investigation since the hydrated salt decomposes on heating to over 33° C. into the anhydrous salt and water. The object

[16] *Zeits. f. Phys.,* 1932, 77, 271.
A5

of the present investigation is therefore to study the effect of binding on the susceptibility of the hydrate.

The initial temperature of the solution was usually about 28° C. The depressions obtained in the Quincke method were noted just at this temperature and then at higher temperatures as the liquid was gradually heated. No changes in the depression were observed as the temperature was raised to above 33° C. The solution was then cooled to the laboratory temperature and no change was observed even when the measurements were repeated over a considerable period (3 to 4 hours) during which interval, the salt should have become a hydrate again. This result definitely indicates that no important change in the electronic configuration occurs on dehydration. The values obtained at different temperatures are tabulated below.

TABLE V.

Concentration 11·6%			Concentration 20·0%			Concentration 25·5%		
Temperature °C.	Depression divisions on scale	χ_t/χ_{2s}	Temperature °C.	Depression divisions on scale	χ_t/χ_{2s}	Temperature °C.	Depression divisions on scale	χ_t/χ_{2s}
28	604·0	1·0000	28	581·0	1·0000	28	556·5	1·0000
31	604·1	1·0002	35	580·5	0·9991	31	557·0	1·0009
35	604·5	1·0008	38	581·0	1·0000	36	557·2	1·0012
40	604·3	1·0005	42	581·2	1·0003	40	556·8	1·0005
44	605·0	1·0016	44	581·0	1·0000	42	557·0	1·0009

The measurements have been made at three different concentrations. It is found that the value of the susceptibility remains constant for any concentration when the temperature is raised from 28° C. to 44° C. It is thus clear that whether water exists as water of crystallisation with sodium sulphate or separately, the susceptibility is the same.

The results obtained with the Curie balance also supports the same conclusions. The solid was sealed in a bulb and the deflection was noted. The bulb was then heated gently to temperatures above 33° C. and the deflection taken again. A large number of readings were taken. About 66 to 70 milligrams of the hydrated crystal gave deflections varying from 8 to 9 centimetres. Heating to temperatures above 33° C. did not produce any change in the deflection and hence in the mass susceptibility of the solid when the water of hydration became free and the salt was anhydrous.

4. Discussion.

The molecular susceptibility of a polyatomic molecule without a resultant spin is represented according to Van Vleck[17] by the expression

$$\chi_{mol} = -\frac{Le^2}{6mc^2} \Sigma \bar{r^2} + \frac{2}{3} L \Sigma \frac{|m_0(n';n)|^2}{hv(n';n)}$$

The first term is the well-known term of Langevin while the second is a paramagnetic term independent of temperature and is brought about by the distortion of the electron system due to interatomic forces such as are obtained in diatomic and polyatomic molecules. The substance is diamagnetic or paramagnetic depending on whether the first or the second term is the greater. The second term is however usually small, although the difference between the observed and theoretical values of the susceptibility in the case of some sulphur compounds has been found to be as much as 30 per cent. It is evident from the discussion that the paramagnetic term should vary with the different linkages in the molecule and an additional linkage would mean an increase of this term and hence a decrease in the diamagnetic susceptibility. In solutions, the ions are not free but attach themselves to solvent molecules[18] and thus because of the irregular spacing of a liquid, sufficient asymmetry is brought about resulting in a distortion of the electron system. When a salt is dissolved in a liquid, the binding in the solid state is broken off and at the same time new constraints are brought about because of the attachment of the solvent molecules to the different ions of the salt. The change in susceptibility that ought to be expected therefore when a salt is dissolved in a liquid is due to the difference in the paramagnetic term when in the solid state and in solution. The diamagnetic susceptibility in the solid state would consequently be greater or smaller according as the binding in the solid state is smaller or greater than that in solution. Also if new linkages are formed the system will be affected and the deviation of the susceptibility value will indicate in each case the nature of the linkages involved.

We can also draw attention to the fact that hydration of the ions in solution would indicate greater constraint on the ion and hence the paramagnetic term would become greater resulting in a smaller value for the diamagnetic susceptibility.

In the light of these conclusions, the results for aqueous solutions of sulphuric acid, acetic acid and sodium sulphate are quite interesting. In the case of sulphuric acid-water mixture, we meet with two types of deviations.

[17] Van Vleck, *Theory of Electric and Magnetic Susceptibility*, p. 275.
[18] Fajans, *Trans. Faraday Soc.*, 1927, **23**, 357.

At 91·6 per cent. of the acid, the hydrate $2H_2SO_4, H_2O$ is formed and it gives an increase in the susceptibility value ; whereas the other hydrates give lower values. It is easy to understand the decrease of diamagnetic susceptibility in the case of the higher hydrates ; but the increase in the case of $2H_2SO_4, H_2O$ requires some explanation.

We shall now show that the considerable deformation produced in the SO_4^{--} ion by the hydrogen cations on combination is somewhat reduced by the water molecules and since the paramagnetic term in the above equation is consequently decreased, the diamagnetic susceptibility shows an increase. These assumptions are justified by Raman effect and X-ray data.

Krishnamurthi[19] has studied the effect of the cation on the sulphate group and has come to the conclusion that the smaller cations have greater deforming power. Thus it would appear that H^+ would have a large deforming power on the anion. That there is considerable influence on the Raman frequencies of the anion by the cation and that the smaller cations produce much larger changes are amply borne out by the investigations of Gerlach and Embirikos.[20]

These conclusions receive additional confirmation from the study of the k absorption edges. Lindh[21] has shown that the frequency of the continuous k absorption edge of an element in a compound radical varies with its valency. In the case of positive ions like those of chromium, manganese and iron, the separation increases with the valency. De Broglie[22] and Lindsay and Voorhes[23] have also verified these conclusions. In the case of anions, the absorption edge shifts towards shorter wave-length as their deformations are increased. Introduction of a neutral molecule like water between the positive and negative ions lowers the deformation of the anions on account of increased distance and hence the absorption edge is shifted towards longer wave-length which indicates that the binding has become more loose.

In the magnetic case, it has been noted that the term Δ in the Curie-Weiss law, which is primarily due to interatomic forces, is almost zero for salts of high magnetic dilution such as $MnSO_4(NH_4)_2SO_4 \cdot 6H_2O$ and $Fe_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$. In both cases the term Δ is nearly zero since there are large numbers of ammonia and water molecules which lessen the interatomic forces considerably.[24]

[19] *Ind. Jour. Phys.*, 1930, 513 and 651.
[20] Taken from Kohlrausch, *Smekal-Raman Effect*, p. 204.
[21] *Zeits. f. Phys.*, 1925, 31, 210.
[22] De Broglie, *X-Rays* (Eng. Trans.), p. 75.
[23] *Phil. Mag.*, 1928, 6, 910.
[24] *Theory of Electric and Magnetic Susceptibilities*, p. 304.

The case of $2H_2SO_4$, H_2O can be considered as another example where we have a similar dilution effect. The introduction of water molecules loosens the bond between the hydrogen ions and the sulphate ion and hence decreases the deformation. This is accompanied by an increase in the diamagnetic susceptibility. There is considerable evidence, in fact, in favour of the idea of the penetration of the proton into the chloride ion in the case of HCl.[25] Such a penetration would cause a general contraction more especially of the superficial orbits which contribute the largest share to the diamagnetic susceptibility. The loosening of the bond with water would therefore mean an increase in value.

As contradistinguished from this effect, we have the formation of the other hydrates which result in a decrease in diamagnetism. In these cases the water molecules form compounds with the acid and the distortion produced by them is greater than the loosening effect produced when the water molecules are first introduced. A reference to Fig. 2 also indicates that the law of mass action is applicable to the formation of these hydrates.

In the case of acetic acid-water, a compound is formed and the alteration introduced into the electronic system is so small that there is no sensible deviation from the additivity law. The break-up of the decahydrate of sodium sulphate at temperatures higher than $33°C$. is not also accompanied by any change of susceptibility at any rate to any measurable extent. The nature of combination in this case is no doubt very loose for it is well known that the sodium sulphate crystals give out their water of crystallisation very easily ; on the other hand, sulphuric acid *absorbs* water vapour very strongly to form stable hydrates.

Attention was drawn to the investigations of Cabrera and Fahlenbrach who studied aqueous solutions of potassium iodide and observed changes in the magnetic susceptibility due to different degrees of hydration. They suggest that there is greater hydration at higher temperatures and that there is an increase of susceptibility corresponding to greater deformation of the ion with increasing hydration. One should expect however a decrease in hydration with increasing temperature. Besides it is quite unlikely that hydration is accompanied by an increase in susceptibility, although in the case of $2H_2SO_4$, H_2O there is an increase for reasons mentioned earlier in the paper. Such an explanation in the case of potassium iodide and generally of strong electrolytes seems to be not justifiable since we have no reason to believe that the distortion caused by hydration itself could be less than the crystalline forces in the solid. It is to be recalled that sulphuric acid

[25] Bell, *Phil. Mag.*, 1924, **47**, 549.

is not as strong an electrolyte as the alkaline halides. The condition for electro-valency tending to become covalent is that the anion must be large and the cation must be very small. This condition being fulfilled in the case of the acid, we ought to expect sulphuric acid to be less electrovalent than the salts mentioned. This indeed is the case as evidenced by the comparatively low dissociation factor of the acid. A break-up of the molecule therefore causes the removal of a large deformation.

Hocart[26] however records a larger susceptibility for a salt in solution than the crystalline state. This difference has been explained by Weiss as being due to deformation in the ion and from the *experimental* values of refractivity a correction has been applied. However there seems to be no real difference between the susceptibility as calculated from the solid state and from the state in solution, provided the depolymerisation of water due to the introduction of the electrolyte is taken into account. In the case of KCl and NaCl for which accurate data are available,[26] it is found that an increase of $0 \cdot 0018$ and $0 \cdot 003$ in the susceptibility of water due to depolymerisation (on the addition of 22% and 25% of KCl and NaCl respectively) would explain the increase observed. When due allowance is made for incidental errors, it looks very probable that this increase is due to depolymerisation of water which has been amply borne out by Raman effect data. It may be mentioned here that measurements in solution must be taken at different increasing temperatures and that the value of the solution which is not altered on increase of temperature, must be used in the calculation of ionic values. In that case the value assumed for water must be that in the completely depolymerised state. Calculations not based on these principles may be in error by as much as 4%.

It thus looks improbable that in these case., hydration causes any change in the susceptibility due to deformation of the ions. The results for sodium sulphate and acetic acid show definitely the comparative insensitivity of diamagnetism to small changes. It may be therefore concluded that the observed changes of Cabrera and Fahlenbrach are to be attributed partly to depolymerisation effects of the ions of water. It is interesting to note that Tammann[27] in a recent communication has drawn attention to the changes in ice VI molecule under the altered conditions of internal pressure brought about by the salt and the increasing temperature. More data on different salts in solution are necessary to decide this important issue. A determination of the susceptibility of water at still higher temperatures

[26] *Comp. Rend.*, 1929, **188**, 1151.
[27] *Zeits. f. Phys.*, 1934, **91**, 410.

would throw light on the interesting question of the existence of ice VI molecule.

5. *Summary and Conclusions.*

The magnetic susceptibilities of sulphuric acid-water mixtures have been studied at different concentrations. Above 86% of the acid, the susceptibility values are greater than those given by the additive law while below 86%, the values are lower than the additive values. Maximum deviations were observed at concentrations corresponding to $2H_2SO_4$, H_2O; H_2SO_4, H_2O; H_2O_4, $3H_2O$; H_2SO_4, $6H_2O$ and H_2SO_4, $18H_2O$. These hydrates are also indicated by other physical properties of the mixture. The deviations have been accounted for as being due to increased deformation of the anion in the acid in one case and decreased deformation in the other.

Acetic acid-water mixtures have been investigated at different concentrations. The results show that the additive law is obeyed even at equimolecular concentration of the components although a compound is formed at this composition.

The decahydrate of sodium sulphate has been studied both in the solid state by the Curie method and in solution by the modified Quincke method used for sulphuric acid-water mixtures. When the hydrate was heated to more than 33° C. (at which temperature the water of crystallisation breaks away and the crystal becomes anhydrous) no change of magnetic susceptibility was noted. This suggests that the binding of the water molecules to the sulphate is very loose in contradistinction to the case of the hydrates of sulphuric acid. The theoretical basis for the increase of susceptibility on hydration suggested by Cabrera and Fahlenbrach to explain their results in aqueous solutions of potassium iodide is critically examined and evidence is put forward to show that no such changes are likely to occur.

IRRATIONAL INDEFINITE QUADRATIC FORMS.

By S. Chowla,
Andhra University, Waltair.

1. I have recently proved the

Theorem.[1] *If the c's are not all of one sign and if all the ratios $\dfrac{c_s}{c_t}$ ($s \neq t$) are irrational, we can find integers $n_1, \cdots, n_r$ (not all zero) such that*

$$\left| \sum_{s=1}^{r} c_s n_s^2 \right| < \epsilon$$

where ϵ is an arbitrary positive number and $r \geqslant 9$.

This was deduced from a theorem of Jarnik and Walfisz in the theory of lattice points.

In the same direction we have

Theorem 1. Let $c_1, c_2 > 0$, $\sqrt{\dfrac{c_1}{c_2}}$ irrational,

$$(1) \qquad \sqrt{\frac{c_1}{c_2}} = a_1 + \frac{1}{a_2+} \ \frac{1}{a_3+} \cdots$$

Then, to every positive ϵ, we can find infinitely many pairs of positive integers n_1 and n_2, such that

$$(2) \qquad | n_1^2 c_1 - n_2^2 c_2 | < \epsilon$$

whenever $a_n \neq O(1)$, but not otherwise.

Theorem 2. If the c's are not all of one sign, then for ' almost all ' sets $(c_1, c_2, \ldots, c_r)$ we can find integers $n_1, \ldots, n_r$ (not all zero) such that

$$\left| \sum_{s=1}^{r} c_s n_s^2 \right| < \epsilon$$

where ϵ is an arbitrary positive number and $r \geqslant 2$.

2. Let $\dfrac{p_n}{q_n}$ be the nth convergent to (1).
Then

$$(3) \qquad \left| \frac{p_n}{q_n} - \sqrt{\frac{c_1}{c_2}} \right| < \frac{1}{a_n q_n^2}.$$

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[1] *Jour. London Math. Soc.*, 1934, **9**, 162-63.

Further, (2) requires,

$$(4) \qquad \left| \frac{n_2}{n_1} - \sqrt{\frac{c_1}{c_2}} \right| = 0 \left(\frac{1}{n_1{}^2} \right)$$

From (3) and (4) we obtain Theorem 1, since a_n is unbounded.

To prove Theorem 2, we can assume without loss of generality that c_1 and c_2 have opposite signs. Let $c_1 = b_1 > 0$, $c_2 = -b_2$. Then $b_1 > 0$, $b_2 > 0$.

We know that if

$$\theta = d_1 + \frac{1}{d_2 +} \quad \frac{1}{d_3 + \cdots}$$

where the d's are positive integers, then $d_n \neq O(1)$ for almost all θ. It now follows from Theorem 1 that for almost all (b_1, b_2) we can find positive integers n_1 and n_2 such that

$$(5) \qquad | b_1 n_1{}^2 - b_2 n_2{}^2 | < \epsilon$$

where ϵ is an arbitrary positive number. Hence for almost all sets (b_1, b_2) we have from (5),

$$(6) \qquad | b_1 n_1{}^2 - b_2 n_2{}^2 + c_3.0^2 + c_4.0^2 + \cdots + c_r.0^2 | < \epsilon \; [n_1, n_2 > 0].$$

Hence Theorem 2 follows from (6), as we can take $n_3 = \cdots = n_r = 0$.

If in Theorem 2 we require that the integers $n_1, \cdots, n_r$ are *all different from zero*, then Theorem 2 is no longer easy to prove — there is, however, little doubt that the theorem is true even with this restriction.

POSITIVE DETERMINANTS OF BINARY QUADRATIC FORMS WHOSE CLASS-NUMBER IS 2.

BY M. SURYANARAYANA, B.A. (HONS.), B.ED.

1. Schaffstein[1] has given a list of *positive prime discriminants* of binary quadratic forms whose class-number is 1. His table shows that the majority of *positive discriminants* under 10000 has their class-number equal to 1.

This paper contains a list of *positive prime determinants* [≡ 3(4)] of binary quadratic forms whose class-number is 2. The table extends to positive determinants under 5000, and here again we find that by far the greater majority of determinants considered has the property in question.

2. To test whether $h(d) = 2$ where d is a positive prime $\equiv 3(4)$ and $h(d)$ is the class-number of binary quadratic forms of determinant d, the following test, suggested to me by Dr. S. Chowla, was used (the proof of the ' test ' is implicit in known results) :

Let $d \equiv 3(4)$, $d > 0$. *Let* p *denote any prime factor of* $d - x^2$ $(x < \sqrt{d})$, $p < \sqrt{d}$. *Then* $h(d) = 2$ *if* p *occurs as a ' partial quotient ' in the simple continued fraction for* $\sqrt{d}$.

(1) The following are values of $d < 2000$ for which $h(d) = 2$, the values of d for which $h(d) > 2$ are marked with an asterisk :

7, 11, 19, 23, 31, 43, 47, 59, 67, 71, 79*, 83, 103, 107, 127, 131, 139, 151, 163, 167, 179, 191, 199, 211, 223*, 227, 239, 251, 263, 271, 283, 307, 311, 331, 317, 359*, 367, 379, 383, 419, 431, 439*, 443*, 463, 467, 479, 487, 491, 499*, 503, 523, 547, 563, 571, 587, 599, 607, 619, 631, 643, 647, 659*, 683, 691, 719, 727*, 739, 743, 751, 787, 811, 823, 827, 839*, 859, 863, 883, 887, 907, 911, 919, 947, 967, 971, 983, 991.

1019, 1031, 1039, 1051, 1063, 1087*, 1091*, 1103, 1123, 1151, 1163, 1171*, 1187, 1223*, 1231, 1259, 1279, 1283, 1291, 1303, 1307, 1319, 1327*, 1367*, 1399, 1423, 1427, 1439, 1447, 1451, 1459, 1471, 1483, 1487, 1499, 1511, 1523*, 1531, 1543, 1559, 1567*, 1571, 1579, 1583, 1607, 1619, 1627*, 1663, 1667, 1699, 1723, 1747, 1759, 1783, 1787*, 1811*, 1823, 1831, 1847*, 1867, 1871, 1879, 1907*, 1931, 1951, 1979, 1987, 1999.

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[1] *Math. Annalen*, 1927.

(2) The following are the *only* prime values of $d \equiv 3(4)$ for which $h(d) > 2$, $2000 < d < 5000$:

2027, 2099, 2143, 2207, 2251, 2399, 2459, 2467, 2543, 2659, 2711, 2971, 3023, 3163, 3203, 3251, 3271, 3391, 3719, 3739, 3803, 3967, 4139, 4159, 4271, 4283, 4591, 4651, 4759.

3. Of the 335 primes $d \equiv 3(4)$ upto 5000, 284 primes satisfy $h(d) = 2$, while 51 do not.

I am indebted to Dr. S. Chowla under whose guidance I worked.

ON SUMS OF POWERS.

By Inder Chowla.

1. A non-trivial solution of the equation

$$(1) \quad x_1^k + x_2^k + \cdots + x_m^k = y_1^k + \cdots + y_n^k$$

in positive integers is one in which no x is equal to any y and

$$(x_1, \cdots, x_m, y_1, \cdots, y_n) = 1.$$

When there exists a non-trivial solution of (1) we write

$$(2) \quad (m)^k = (n)^k$$

We use $\beta = \beta(k)$ to denote the least value of n such that (1) has a non-trivial solution with $m < n$, and $\gamma = \gamma(k)$ to denote the least value of n such that (1) has an infinity of non-trivial solutions with $m < n$.

We use $r_{k,s}(N)$ for the number of representations of N as a sum of s positive kth powers (permutation of the bases not being allowed) and $r'_{k,s}(N)$ for the number of primitive representations.

Wright[1] has shown that

Theorem 1. For $3 \leqslant k \leqslant 9$ we have $\gamma(k) \leqslant k$.

Rao[2] has shown that

Theorem 2. For $4 \leqslant k \leqslant 8$ we have $\beta(k) \leqslant k - 1$.

I prove here that

$$(3) \quad \beta(9) \leqslant 8$$

so that we obtain

Theorem 3. For $3 \leqslant k \leqslant 9$ we have $\beta(k) \leqslant k - 1$.

I also find that

Theorem 4.
$$\text{(i)} \quad (5)^7 = (5)^7$$
$$\text{(ii)} \quad (4)^7 = (6)^7$$
$$\text{(iii)} \quad (7)^9 = (7)^9$$

Of these relations (ii) and (iii) are new, while (i) has also been established by Rao in a paper to appear in *Mathematische Zeitschrift*.

[1] *Journ. London Math. Soc.*, 1935, **10**.

[2] The cases $k=5, 6, 8$ in *Journ. London Math. Soc.*, 1934, **9**, 170-71, 172-73 and *Mathematische Zeitschrift*, 1934, **39**, 240-43. The case $k=7$ in a paper to appear in *Mathematische Zeitschrift*.

Further, Wright[3] has shown that

Theorem 5. *For* $3 \leqslant k \leqslant 9$,
$$r'_{k,k}(N) \geqslant 2 \text{ is true for infinitely many N.}$$

From Theorem 4, (i) and (iii) we deduce that

Theorem 6. *For* $k = 7$ *and for* $k = 9$,
$$r'_{k,k-1}(N) \geqslant 2 \text{ is true for infinitely many N.}$$

2. Our Theorem 2 is a special case of

Theorem 7. *For* $r = 1, 3, 5, 7, 9$ *we have*
$$1^r + 4^r + 7^r + 32^r + 33^r + 44^r + 47^r + 48^r$$
$$= 14^r + 26^r + 39^r + 42^r + 46^r + 49^r$$

In particular, we have,
$$(6)^9 = (8)^9$$
so that $\beta(9) \leqslant 8$.

The results of Theorem 4 are special cases of the following:

(4) $\quad 1^r + 5^r + 13^r + 39^r + 51^r + 59^r$
$\qquad = 23^r + 33^r + 55^r + 57^r \quad [r = 1, 3, 5, 7]$.

(5) $\quad 7^r + 11^r + 19^r + 61^r + 69^r + 91^r + 93^r$
$\qquad = 1^r + 13^r + 25^r + 55^r + 75^r + 87^r + 95^r \, [r = 1, 3, 5, 7, 9]$.

(6) $\quad 15^r + 27^r + 51^r + 61^r + 69^r$
$\qquad = 13^r + 31^r + 47^r + 65^r + 67^r \quad [r = 1, 3, 5, 7]$.

3. All our results are obtained by " Tarry's process ". A complete derivation of the results will appear elsewhere. Our method also proves

Theorem 8. *We have* $\beta(17) \leqslant 31$.

[3] *Loc. cit.*

A PRACTICAL FINANCIAL TRANSACTION.

By G. S. Diwan
AND
V. V. Narlikar.

A MESSENGER prize of the Institute of Actuaries, London, was awarded in 1933, for the first time, to an Indian, Mr. D. P. Misra,[1] for demonstrating that multiplicity of the rate of interest is possible for a financial transaction and for propounding the need of a criterion by which a unique rate of interest may be determined in such cases of ambiguity. It had been taken for granted before, presumably without any mathematical proof to support it, that a financial transaction admits only one rate of interest; and hence the examples given by Misra in which two or more rates of interest become possible evoked much interest among the leading actuaries of the world.

A precise definition of a practical financial transaction must first be stated as it is intended to treat it here mathematically. The definition proposed by us is this: throughout a practical financial transaction, on the basis of a uniform rate of interest, the original creditor always remains the creditor, and the original debtor always the debtor and that the two are quits only when the transaction comes to an end. This definition is not satisfied by the examples of Misra wherein multiplicity arises.

The definition of a practical financial transaction may now be expressed as a set of mathematical conditions. Let a financial transaction start with A lending a sum a_0 to B on a certain date, say, January 1st, 1935. If A is the creditor $a_0 > 0$. Exactly a year from that date A lends another sum a_1 to B or B returns a sum $-a_1$ to A according as a_1 is +ve or —ve. If $y\%$ is the rate of interest we may denote $1 + y/100$ by x. According to the definition of a practical transaction $a_0 x + a_1 \geq 0$ on January 1st, 1936. If $a_0 x + a_1 = 0$ the transaction becomes complete and the rate of interest is $10^2 (-a_1/a_0 - 1)\%$. If the transaction is not complete on January 1st, 1936, let it be completed exactly n years after the initial payment. If at the end of the rth year from the beginning A lends a_r to B or B returns $-a_r$ to A according as a_r is +ve or —ve, then the following algebraic conditions hold good :

[1] *Journal of the Institute of Actuaries*, Vol. 64, pp. 71-97. *Vide* also Dr. Steffensen's paper in the same volume.

$$f_n(x) \equiv a_0 x^n + a_1 x^{n-1} + \cdots + a_n = 0$$
$$f_r(x) \equiv a_0 x^r + a_1 x^{r-1} + \cdots + a_r > 0, \, r = 0, 1, \ldots, n-1 \Big\} \quad \cdots \quad \text{(I)}$$

If $a_r = 0$ it means of course that no payment occurs between the parties at the end of the rth year. The uniqueness will be established when we prove that if there exists a root of $f_n(x) = 0$, say, $x = a > 1$ satisfying all the conditions (I) then there cannot exist $\beta > 1$ satisfying $f_n(x) = 0$. This is an algebraic problem and we proceed to prove now a slightly more general problem :

If $a \geqslant 0$ satisfies all the conditions (I) then $f_n(x) = 0$ has no other positive root.

We have

$$f'_n(x) = [f_{n-1} + x f_{n-2} + \cdots + x^r f_{n-r-1} + \cdots + x^{n-1} f_0] \quad \cdots \quad \cdots \quad \text{(II)}$$

It is clear from (II) that at $x = a$ $f'_n(a)$ is positive. As in (II) $f'_{n-1}(x)$, $f'_{n-2}(x)$, etc., may also be expressed by similar finite series. Hence $f'_n(a)$, $f'_{n-2}(a)$, etc., are all positive. It follows therefore that $f''_n(x)$ is +ve at $x = a$. Proceeding in this manner it becomes clear that $f'''_n(a), f^{IV}_n(a), \cdots f^n_n(a)$ are all positive and that the higher derivatives vanish. Using the Taylor polynomial expansion we find that $f_n(x) > 0$ for $x > a$ and that $f_n(x) = 0$ cannot therefore have another root greater than a.

We can also show that there cannot exist another root β of $f_n(x) = 0$ such that $0 < \beta < a$. For obviously $f_0(\beta) = f_0(a) > 0$. Hence $a f_0(a) + a_1 > \beta f_0(\beta) + a_1$ or $f_1(a) > f_1(\beta)$. Since $f_1(a) > 0$ we obtain similarly $f_2(a) > f_2(\beta)$. Applying this process continuously the conclusion is reached that $f_n(a) > f_n(\beta)$. But $f_n(a) = 0$. Hence β cannot be a root of $f_n(x) = 0$.

It is this algebraic proposition that supplies a proof of the uniqueness of the rate of interest in a practical financial transaction. We have considered the simple case where payments and returns are made at yearly intervals, but a more sophisticated case where payments and returns are made at any intervals which however can be expressed by commensurable integers reduces to a similar algebraic problem to which the result of the general proposition established becomes applicable.

Incidentally the general algebraic proposition is also valid if some, but not all, $f_r(x)$ vanish at $x = a$. The case is certainly trivial where all $f_r(x)$ vanish at $x = a$. It is also evident that the root a is not repeated except possibly when $a = 0$.

When this investigation was being carried out a new theorem in the theory of equations occurred to us. In view of the close connection between the mathematical arguments of this paper and this theorem it may not be

out of place to state and prove it here briefly. Following the notation of the paper we state the theorem as follows :

If $f_r(x) = 0$ has p roots each greater than a, then there are at least p changes of sign in the series $f_0(a), f_1(a), \cdots, f_r(a)$ where $a \geqslant 0$.

Consider $(x - \beta) f_r(x) = \psi_{r+1}(x)$ for $\beta > a$. If $\psi_{r+1}(x) \equiv b_0 x^{r+1} + b_1 x^r + \cdots + b_{r+1}$ we may define $\psi_t(x) \equiv b_0 x^t + b_1 x^{t-1} + \cdots b_t$ for $t = 0, 1, \cdots r$; so that $\psi_t(a) = (a - \beta) f_{t-1}(a) + a_t$, $\psi_0(a) = f_0(a)$ and $\psi_{r+1}(a) = (a - \beta) f_r(a)$. Now consider the signs in the two series :

$$f_0(a) \quad f_1(a) \cdots \cdots f_r(a)$$
$$\psi_0(a) \quad \psi_1(a) \cdots \cdots \psi_{r+1}(a).$$

The two series begin with the same sign and end with opposite signs. Corresponding to a change of sign from $f_{t-1}(a)$ to $f_t(a)$ the sign of $\psi_t(a)$ agrees with the latter ; and corresponding to a continuation of sign in the first from $f_{t-1}(a)$ to $f_t(a)$ the sign of $\psi_t(a)$ remains ambiguous. When a zero value comes in either series we may take the left hand sign.

Hence it follows that the ψ-series has at least one more change of sign than the f-series. If the f-series is perfectly general so also is the ψ series, the increase in changes of sign in the latter series being due to the fact that $\psi_{r+1}(x) = 0$ has one more root (*viz.*, β) exceeding a than $f_r(x) = 0$. This completes the proof of the theorem, which may be regarded as a generalisation of Descartes' rule of signs.

Summary.

A definition of a practical financial transaction is given and it is proved straight from the definition that a practical transaction admits only one rate of interest. The transactions considered by Misra in his paper are thus shown to be only mathematically possible. Two new algebraic theorems are stated and proved.

CHEMISTRY OF β-ARYL GLUTACONIC ACIDS.

Part II. Condensations with Phenolic Ethers.

By G. R. Gogte, M.Sc.

(*From the Department of Organic Chemistry, Indian Institute of Science, Bangalore.*)

Introduction.

In Part I of this series,[1] the β-(β-methoxy-naphthyl)-glutaconic acid was found to be the first case of a glutaconic acid of the β-aryl type to be separated in geometrically isomeric forms, and later on[2] it was found possible to transform all the naphthol substituted glutaconic acids into their geometrical isomerides. In a search for more glutaconic acids belonging to the β-aryl type exhibiting geometrical isomerism, the author happened to condense *p*-cresol-ethylether with acetone-dicarboxylic acid. This condensation, however, gave only one form of a β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (I), besides two other new acids melting at 205° C. and 232° C. These acids were found to belong to an entirely new type of compounds which could be derived from glutaconic acids, and form the subject of the present investigation.

The equivalents and empirical formulæ of these acids were of a grade differing from the glutaconic acid by about one molecule of *p*-cresol-ethylether, and this combined with their monobasic character, suggested their probable formation by interaction of the cresolether with one of the carboxylic groups of the glutaconic acid during the reaction. However, when it was attempted to condense the glutaconic acid (I) with *p*-cresol-ethylether in the presence of concentrated sulphuric acid as in the original reaction, no reaction was observed to proceed in the expected manner. Similar condensation products of phthalic and succinic acids with aromatic hydrocarbons or phenolic ethers have been obtained in the past from their anhydrides, by the application of Friedel and Crafts' reaction.[3] This reaction

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[1] Gogte, *Proc. Indian Acad. Sci.*, A, 1934, 1, 48 (0).

[2] Gogte, *Proc. Indian Sci. Congress*, 1935, Abst. No. 115.

[3] Pechmann, *Ber.*, 1880, 13, 1612; Burker, *A.ch.* (5) 26, 435, 499; Nourrison, *Ber.*, 19, 2103; Ullmann and Schmidt, *Ber.*, 52, 2098; Bentley, Gardner and Weizmann, *J.C.S.*, 1907, 1626.

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was found unworkable in the present case, in that, the glutaconic anhydride, being an unsaturated compound, was itself attacked by aluminium chloride. Moreover, zinc chloride or phosphorus pentoxide could not be used here as condensing agents, for they transformed the glutaconic acid into its anhydride, which prevented further reaction. The desired condensation, however, was found to get effected, in the presence of 80% sulphuric acid, appreciable quantities of the acids m.p. 205°C. and 232°C. being formed.

The monocarboxylic acid m.p. 205°C., however, did not absorb any bromine from bromine water, did not decolourise alkaline potassium permanganate solution and gave no semicarbazone, thus indicating an absence of any double bond or a ketonic group in itself. This went against the above supposition about its formation. When treated with 80% sulphuric acid this monocarboxylic acid gave the known 6-methyl-coumarin-4-acetic acid[4] (VI), while the action of concentrated sulphuric acid produced together with this coumarin acid, a neutral compound m.p. 181°C. with an empirical formula showing a loss of one molecule of ethyl alcohol during its formation. This neutral compound, on boiling with caustic alkalies, went slowly in solution, and on subsequent acidification produced an acid, which decomposed at 110°C. and gave back the neutral compound. This new acid was also extremely unstable giving back the original neutral compound even on simple crystallisation ; this suggested an existence of a lactone ring similar to that in the coumarins, in the latter. Hydrolysis and subsequent ethylation of the neutral compound to prevent this lactone-ring closure, yielded, instead of the original monocarboxylic acid m.p. 205°C., a new dicarboxylic acid m.p. 219°C. with an empirical formula showing an excess of one molecule of ethyl alcohol over the monocarboxylic acid. This indicated the presence of a lactone ring also in the monocarboxylic acid m.p. 205°C. which was proved by its transformation into the dicarboxylic acid m.p. 219°C. by hydrolysis and ethylation. On the other hand, the dicarboxylic acid m.p. 219°C. was also found to give, by the action of sulphuric acid, a mixture of the monocarboxylic acid m.p. 205°C., the neutral compound m.p. 181°C. and the 6-methyl-coumarin-4-acetic acid (VI).

It was therefore concluded that the dicarboxylic acid m.p. 219°C. was the primary product of the reaction formed evidently by the addition of one molecule of p-cresol-ethylether to the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (I) ; the monocarboxylic acid m.p. 205°C. (II) and the neutral compound m.p. 184°C. (V) being its mono and dilactones. Such direct additions of aromatic hydrocarbons or phenolic ethers to a double bond,

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[4] Dey, *J.C.S.*, 1915, 1636.

are known to happen in the case of styrols,[5] and cinnamic acid.[6] Thus here the dicarboxylic acid m.p. 219° C. can be represented by either of the structures (III) and (IV), according to which carbon atom along the double bond, the nucleus of the phenolic ether is attached. The symmetrical structure (III) designating the dicarboxylic acid as $\beta\beta'$-(22'-diethoxy-55'-dimethyl-diphenyl)-glutaric acid, is the more probable one, because an acid of the structure (IV) would give in addition to the 6-methyl-coumarin-4-acetic acid (VI), an acid of the formula (VII) by the action of sulphuric acid ; no such acid was detected. These reactions and all the products could be represented as :

monocarboxylic acid m.p. 232°C.
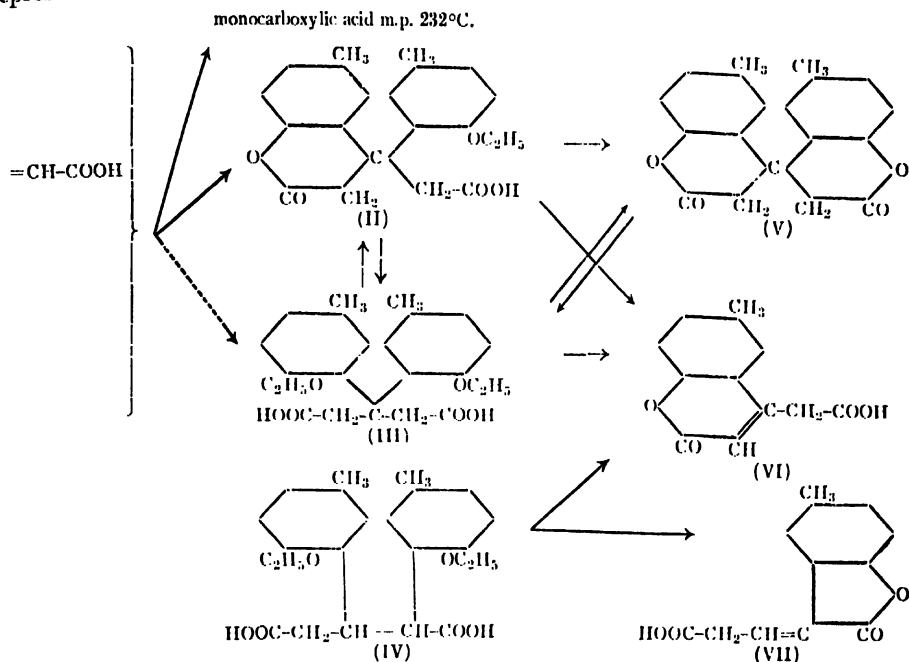
β-Substituted glutaric esters are known to condense with oxalic ester, but the reaction is more difficult when there are two methyl groups in the β-position.[7] The esters of the present glutaric acid could not be made to condense with oxalic ester. This may be due to the fact that there are two much heavier phenolic ether groups in the β-position, which cause a kind of steric hindrance as in the case of $\beta\beta$-dimethyl-glutaric esters.

[5] König, *Ber.*, **23**, 3145; Krämer and Spilker, *Ber.*, **23**, 3169, 3269.

[6] Libermann and Hartmann, *Ber.*, **24**, 2582; **25**, 957.

[7] Dieckemann, *Ber.*, 1930, **32**; Komppa, *Annalen*, **368**, 126.

In the other monocarboxylic acid m.p. 232° C., the absorption of bromine from bromine water, the instantaneous decolourisation of alkaline potassium permanganate solution, and the easy formation of semicarbazone indicated the existence of unsaturation and a ketone group. This showed that it had been formed by the elimination of a molecule of water between one of the carboxylic groups of the glutaconic acid (I) and the nuclear hydrogen atom of p-cresol-ethylether. A similar monocarboxylic acid melting at 252° C. had been obtained previously by the condensation of p-cresol-methylether with acetone-dicarboxylic acid.[8] It has now been found possible to synthesise this also by the condensation of p-cresol-methylether with β-(2-methoxy-5-methyl-phenyl)-glutaconic acid (VIII) in the presence of 80% sulphuric acid. It was observed that no glutaric acid like (III) was formed in either of these condensations ; the compound could however be synthesised from the dilactone (V) by hydrolysis and methylation. This monocarboxylic acid m.p. 252° C. could be designated by either the structure (IX) or (X), depending upon which of the carboxylic groups of the glutaconic acid had taken part in the reaction.

By the action of 80% sulphuric acid, the monocarboxylic acid m.p. 252° C. produced an indone-acetic acid m.p. 218° C. by the loss of one molecule of p-cresol-methylether, which was identical with the one described by Gogte and Limaye[8] ; but the action of concentrated sulphuric acid yielded, in addition to the indone-acetic acid, a neutral compound m.p. 214° C. by a loss of water caused by the internal condensation of the carboxylic group with the ring. If the structure (IX) be assumed for the monocarboxylic acid, the indone-acetic acid will have the formula (XI) and the neutral compound (XIII). Then the decarboxylation product (XII) of the indone-acetic acid will not contain a reactive-CH_2 CO group in the ring, but such a group will be present in the neutral compound. If, on the other hand the structure (X) represents the monocarboxylic acid, the situation will be exactly reversed and thus a decision between these two alternative formulæ (IX) and (X) can easily be made by examining whether the decarboxylation product of the indone-acetic acid or the neutral compound m.p. 214° C. contains a reactive methylene group by condensing with aromatic aldehydes.[9] It was observed that the neutral compound m.p. 214° C. (XIII) easily condensed with benzaldehyde whereas the decarboxylation product (XII) remained inert, thus supporting the formula (IX) for the monocarboxylic acid m.p. 252° C. This structure is further supported by the observation that

[8] Gogte and Limaye, *J. Univ. Bom.*, **3**, Part II, 135.

[9] Compare Kipping, *J.C.S.*, 1894, **65**, 492.

the esters of the monocarboxylic acid also contained no reactive-CH_2-CO– group condensing with benzaldehyde, as would be the case if formula (X) were correct. In the formula (IX), the carboxylic group of the glutaconic acid is represented as attached at the ortho-position to the methoxy group of the *p*-cresol-methylether, as this is the only reactive position in the nucleus. The monocarboxylic acid m.p. 252° C. can therefore be designated as 2-2′-dimethoxy-5-5′-dimethyl-chalkone-*a*-acetic acid, and the indone-acetic acid as 7-methoxy-4-methyl-3-keto-indene-acetic acid. The fact that in the formation of these acids, the carboxylic group of the glutaconic acid (VIII) situated at the end of a conjugated system of double bonds, has taken part in the reactions, is in harmony with its increased reactivity. These reactions and the above-mentioned products are represented as :

(VIII) → (IX) or (X)

↙ ↘

(XI) → (XII)   (XIII)
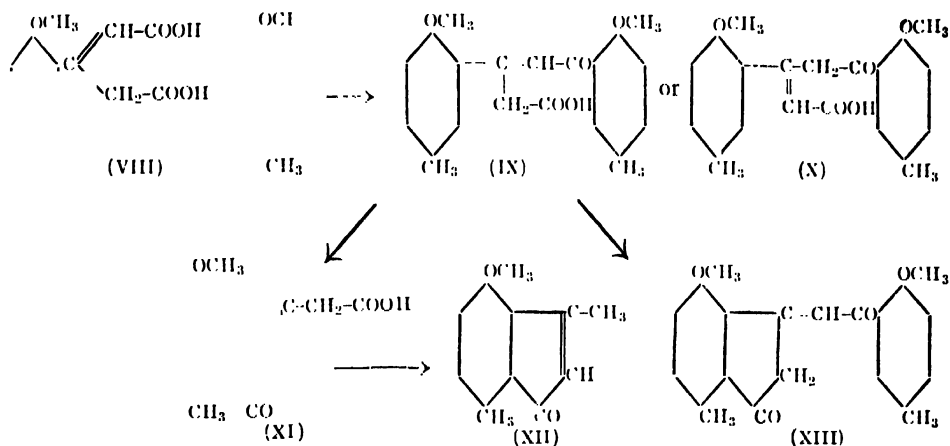
The corresponding monocarboxylic acid m.p. 232° C. obtained in the condensation of *p*-cresol-ethylether, gave by the action of sulphuric acid, an indone-acetic acid m.p. 216° C. and a neutral compound m.p. 165° C. This indone-acetic acid could not however be obtained from the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (I) by the action of sulphuric acid, the 6-methyl-coumarin-4-acetic acid being the sole product in this case.

Conclusion.

Pechmann's condensation consists primarily in the elimination of a molecule of water between the enolic hydroxylic group of the β-ketonic ester and the nuclear hydrogen atom of the phenol, whereas in the Simonis' reaction, it is the carboxylic group of the β-ketonic ester that eliminates water with the latter. In the present condensations of *p*-cresol-methyl and ethyl-ethers with acetone-dicarboxylic acid, both these reactions apparently happen simultaneously to produce the chalkone-acetic acids, and thus they

can be looked upon as a combination of Pechmann's and Simonis' reactions. Similarly the glutaric acid condensation can be called a combination of Pechmann's and Krämer's reaction.

The β-(2-methoxy-5-methyl-phenyl)-glutaconic acid (VIII) by the action of sulphuric acid, produces the indone-acetic acid (XI) as well as the 6-methyl-coumarin-4-acetic acid (VI), whereas the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (I) gives only the latter coumarin acid, no trace of any indone-acid being obtained. The chalkone-acetic acids m.p. 252° C. and 232° C. (IX) on the other hand, give only the indone-acids (XI) under these circumstances, and not even a trace of the coumarin acid. It thus appears that in the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (I) and in both the chalkone-acetic acids, there is a restricted rotation round the bond joining the β-carbon atom of the glutaconic acid to the phenolic ether (shown in thick line). Consequently the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid can be represented only by the formula (XIV) and the chalkone-acetic acid by (XVI). Thus it must be presumed that in the condensation of this glutaconic acid with p-cresol-ethylether, the formation of the chalkone-acetic acid has been preceded by the change of the glutaconic acid from the structure (XIV) to (XV) which alone is capable of producing the indone-acid.

(R = OCH₃ or OC₂H₅.)
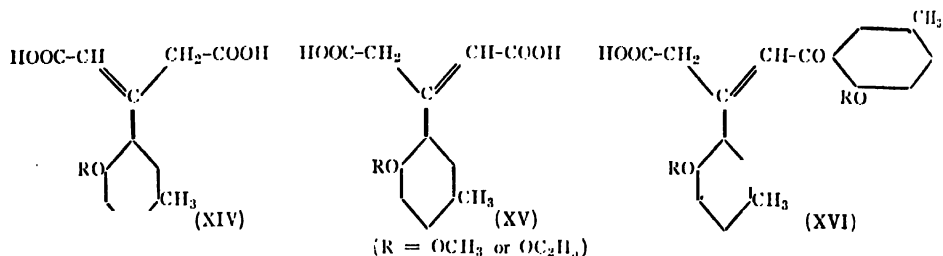
This great difference in the resulting condensation products, caused by the mere replacement of the methoxy group in the p-cresol-methyl-ether by ethoxy group, is striking.

Experimental.

Condensation of p-cresol-ethylether *with acetone-dicarboxylic acid.*—Citric acid (200 g.) was finely powdered and covered with concentrated sulphuric acid (240 c.c.). The mixture was shaken well and fuming sulphuric acid (20 per cent. SO_3 ; 80 c.c.) was gradually added. Immediately a vigorous reaction commenced with frothing and evolution of carbon monoxide, which was completed by heating on a water-bath at 60°C. with shaking at intervals, till a clear orange solution was obtained. It was cooled in a freezing mixture to a temperature of about 2-3° C. and p-cresol-ethylether (65 c.c.) was gradually added with shaking. After keeping at this temperature

for $3\frac{1}{2}$ hours, the reaction mixture was poured on 1000 g. of cracked ice, when the sticky mass thus separated turned into a brittle cake on keeping overnight. This was filtered, dissolved in dilute sodium carbonate solution, this solution washed with ether to remove any unchanged *p*-cresol-ethyl-ether and other neutral impurities and acidified. The semisolid mixture of acids thus obtained became granular on rubbing and keeping overnight with water. Yield 47 g.

β-(2-*ethoxy*-5-*methyl*-*phenyl*)-*glutaconic* *acid*.—The above mixture of acids was treated with 4 litres of boiling water and filtered, when the filtrates on cooling deposited crystals of the glutaconic acid. It was recrystallised from water as colourless short rods m.p. 153° C. (decomp.). On treating with concentrated sulphuric acid at 60° C., or 80% sulphuric acid overnight, it gave only the 6-methyl-coumarin-4-acetic acid m.p. 180° C. (decomp.) (Found : Eq = 132 ; C = 63·44% ; H = 6·6% ; $C_{14}H_{16}O_5$ requires Eq = 132 ; C = 63·6% ; H = 6·66%).

The *hydroxy-anhydride* crystallised in colourless needles from benzene, m.p. 112° C. It titrates as a monobasic acid and gives phenolic colouration with ferric chloride in cold alcoholic solution (Found : Eq = 243 ; C = 68·15% ; H = 5·6% ; $C_{14}H_{14}O_4$ requires Eq = 246 ; C = 68·29% ; H = 5·69%).

The *semianilide*, prepared from the hydroxy anhydride and aniline in benzene solution, crystallised from 60% aqueous methyl alcohol in hexagonal plates m.p. 136° C. It decomposed after 150° C. temperature to give the anil described below (Found : C = 70·5% ; H = 6·1% , $C_{20}H_{21}O_4N$ requires C = 70·8% ; H = 6·16%).

The *hydroxy-anil* crystallised from 80% alcohol in yellowish silky needles m.p. 163° C. (Found : C = 74·5% ; H = 5·80% ; $C_{20}H_{19}O_3N$ requires C = 74·76% ; H = 5·90%).

The monolactone of ββ'-(22'-*diethoxy*-55'-*dimethyl*-*diphenyl*)-*glutaric acid*. —The water insoluble residue in the above (about 15 g.), on crystallisation from alcohol, melted between 190 200° C. This was esterified by alcohol and sulphuric acid and the resulting mixed esters were, by fractional crystallisation from 80% methyl alcohol, separated into two fractions, the more insoluble one melting at 124° C., and the other at 110–115° C. Hydrolysis of the former gave the monolactonic acid which crystallised from alcohol in stout colourless needles, m.p. 205° C. yield 8 g. The acid titrated as monobasic acid and gave an insoluble barium salt in the cold. The acid was soluble in alcohol, acetic acid, acetone, and insoluble in water, benzene, petrol or chloroform (Found : Eq = 356 ; C = 71·06% ; H = 6·12% ; $C_{21}H_{22}O_5$ requires Eq = 354 ; C = 71·2% ; H = 6·2%).

The *ethyl-ester* crystallised from methyl alcohol in colourless hexagonal rods m.p. 124° C. (Found : C=72·1% ; H=6·72% ; $C_{23}H_{26}O_5$ requires C=72·25% ; H=6·8%).

The 22'-diethoxy-55'-dimethyl-chalkone-a-acetic acid.—The more soluble fraction of the mixed ethyl esters melting between 110–115° C., was hydrolysed by alcoholic potash, and the resulting mixture of acids melting between 195 215° C., was dissolved in hot 15% sodium hydroxide solution. On cooling the solution gradually, colourless shining leaflets of a sodium salt separated, which were collected at the pump under strong suction, washed with small amounts of 10% sodium carbonate solution, and acidified in cold by hydrochloric acid. The acid coming out as a fine precipitate was filtered and crystallised from a large amount of alcohol in colourless rectangular plates m.p. 232° C. yield 2 g. The acid is soluble in acetone, sparingly so in alcohol and acetic acid, and insoluble in other organic solvents. It gave an insoluble barium salt in the cold. Its solution in alkali instantaneously decolourised potassium permanganate solution. Its esters did not condense with aromatic aldehydes (Found : C 72·00% ; H=6·7% ; Ba=14·70% ; $C_{23}H_{26}O_5$ requires C=72·25% ; H 6·8% ; $(C_{23}H_{25}O_5)_2Ba$ requires Ba=15·25%).

The *ethyl-ester* prepared by alcohol and sulphuric acid, crystallised from 80% methyl alcohol in colourless parallelogramic plates, m.p. 133° C. (Found : C 72·9% ; H=7·24% ; $C_{25}H_{30}O_5$ requires C 73·2% ; H 7·32%).

Semicarbazone was prepared by refluxing the acid with semicarbazide hydrochloride and sodium acetate in alcoholic solution for 3 hours. On cooling, the semicarbazone separated and when recrystallised from alcohol melted at 264° C. (decomp.) (Found : C 65·3% ; H=6·4% ; $C_{24}H_{29}O_5N_3$ requires C 65·6% ; H=6·6%).

Semicarbazone of the ethyl ester was obtained by refluxing the reactants in alcoholic solution for 7 hours. It crystallised from alcohol in needles m.p. 171° C. (decomp.) (Found : C 66·6% ; H=6·85% ; · $C_{26}H_{33}O_5N_3$ requires C=66·8% ; H=7·06%).

Condensation of β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid with p-cresol-ethylether.—The recrystallised glutaconic acid (10 g.) was finely powdered and dissolved in previously ice-cooled 80% dilute sulphuric acid—1 vol. water : 4 vol. sulphuric acid—(50 c.c.). The *p*-cresol-ethylether (20 c.c.) was then added gradually with shaking at intervals. On continuing the shaking until the two layers disappeared—about 2 hours—the clear orange solution was allowed to stand at the room temperature for 20 hours. This reaction mixture was then poured on 200 g. of crushed ice, when a sticky

mass separated which became granular on keeping overnight. It was filtered, taken up in dilute sodium carbonate, the solution filtered, washed with ether to remove the excess of *p*-cresol-ethylether, and acidified by hydrochloric acid. The mixed acids were filtered and treated with 400 c.c. of boiling 10% aqueous acetic acid, then with 100 c.c. of boiling water, filtered, and dried. Yield of mixed acids 3·5 g. This was esterified by alcohol and sulphuric acid, and separated into the monolactonic acid m.p. 205° C. yield 2 g., and the chalkone-acetic acid m.p. 232° C. yield 1 g. ; exactly as above. The acetic acid filtrate on cooling gave the 6-methyl-coumarin-4-acetic acid.

Action of 80% sulphuric acid on 22'-diethoxy-55'-dimethyl-diphenyl-chalkone-a-acetic acid : Formation of 7-ethoxy-4-methyl-3-keto-indone-acetic acid.—The chalkone-acetic acid (2·5 g.) was finely powdered and dissolved in 80% sulphuric acid by rubbing and warming a little. The red fluorescent solution on keeping over-night at the room temperature was poured in water, the precipitate taken up in dilute sodium carbonate, filtered and acidified. The acid was crystallised from alcohol in short yellow needles m.p. 216° C. (decomp.) ; yield 1 g. The acid is sparingly soluble in alcohol and acetic acid and insoluble in water, benzene, petrol or chloroform. It titrates as a monobasic acid and gives intensely coloured solutions with caustic alkalies. This indone acid could not be obtained from the β-(2-ethoxy-5-methyl-phenyl)-glutaconic acid (Found : C = 68·21% ; H = 5·65% ; $C_{14}H_{14}O_4$ requires C = 68·3% ; H = 5·7%).

The *ethyl-ester* prepared from the acid by alcohol and sulphuric acid crystallised from 80% alcohol as yellow flat needles m.p. 169° C. (Found : C = 69·8% ; H = 6·45% ; $C_{16}H_{18}O_4$ requires C = 70·0% ; H = 6·57%).

The *semicarbazone* was obtained by refluxing the reactants in alcoholic solution for 2 hours. Light needles m.p. 247° C. (decomp.) (Found : C = 59·23% ; H = 5·47% ; $C_{15}H_{17}O_4N_3$ requires C = 59·4% ; H = 5·6%).

The *semicarbazone of the ethyl ester* crystallised from alcohol in yellow needles m.p. 208°C. (decomp.) (Found : C = 61·51% ; H = 6·25% ; $C_{17}H_{21}O_4N_3$ requires C = 61·63% ; H = 6·34%).

The neutral compound from the above chalkone-acetic acid.—This was obtained by treating the chalkone-acetic acid m.p. 232° (2 g.) with concentrated sulphuric acid (10 c.c.) at a temperature of 60° C. for 1 hour. The precipitate obtained on pouring the resulting fluorescent solution in water gradually became granular on keeping for few hours. It was filtered, treated with dilute sodium carbonate solution, and the insoluble portion was crystallised from methyl alcohol in rectangular plates m.p. 165° C. yield 1·5 g.

(Found :   C $=75 \cdot 5\%$;   H $=6 \cdot 46\%$; $C_{23}H_{24}O_4$ requires C $=75 \cdot 8\%$;   H $=$ $6 \cdot 6\%$).

The *semicarbazone* was obtained by refluxing the neutral compound in alcoholic solution with the reagents for 3 hours, and crystallised from alcohol in parallelogramic plates m.p. 245° C. (decomp.) (Found : C $=63 \cdot 2\%$; H $=6 \cdot 3\%$; monosemicarbazone $C_{24}H_{27}O_4N_3$ requires C $=68 \cdot 4\%$; H $=6 \cdot 4\%$; disemicarbazone $C_{25}H_{30}O_4N_6$ requires C $=62 \cdot 76\%$; H $=6 \cdot 28\%$).

Action of sulphuric acid on the monolactonic acid m.p. 205° C. : *Formation of the dilactone of ββ'-(22'-diethoxy-55'-dimethyl-diphenyl)-glutaric acid.*—The recrystallised monolactonic acid (5 g.) was dissolved in concentrated sulphuric acid (20 c.c.) and the solution heated at a temperature of 60° C. for one hour. On pouring in 100 c.c. of ice water and keeping overnight, the crystalline precipitate which separated was filtered and treated with boiling dilute sodium carbonate. The sodium carbonate filtrates on acidification gave the 6-methyl-coumarin-4-acetic acid (yield 1 g.) m.p. 180° C. (decomp.). The neutral dilactone insoluble in sodium carbonate was washed with water and crystallised from 100 c.c. methyl alcohol in colourless pyramids m.p. 184° C. (yield 3 g.) (Found : C $=73 \cdot 90\%$; H $=5 \cdot 14\%$; $C_{19}H_{16}O_4$ requires C $=74 \cdot 00\%$; H $=5 \cdot 2\%$). Action of 80% sulphuric acid on the monolactonic acid overnight produced only the 6-methyl-coumarin-4-acetic acid, and no neutral dilactone. The dilactone slowly went in solution in boiling caustic alkalies, which on acidification carefully after cooling in ice gave an acid, which decomposed at 110° C. and produced the original dilactone. This acid gave intense phenolic colouration with ferric chloride in cold alcoholic solution which disappeared on warming. All the attempts to purify this acid were rendered futile, each time the dilactone being the resulting product.

Hydrolysis and ethylation of the dilactone m.p. 184° C.: *Formation of ββ'-(22'-diethoxy-55'-dimethyl-diphenyl)-glutaric acid.*—The neutral dilactone (1·5 g.) was finely powdered and refluxed with (30 c.c.) of 25% sodium hydroxide solution, when it gradually dissolved. The pale yellow solution was filtered and heated on a small flame just to boiling, while freshly distilled diethyl-sulphate (6 c.c.) was gradually run in during about 20 minutes ; shaking the reaction mixture at frequent intervals. The resulting liquid was refluxed for 15 minutes, when the diethyl ester of the glutaric acid separated as a yellow oil, which solidified to a colourless crystalline mass on cooling to the room temperature. It was filtered, dried in a vacuum over calcium chloride, and crystallised from methyl alcohol, obtaining thus (1·2 g.) of colourless parallelogramic plates m.p. 82° C. Hydrolysis of the diethyl ester with alcoholic potash furnished the glutaric acid which crystallised from alcohol in colourless parallelogramic plates, m.p. 219° C.

(decomp.). Some more of the glutaric acid was obtained by cooling the above alkali filtrates in ice, when its sodium salt separated as colourless silky needles. These were filtered, treated with boiling dilute hydrochloric acid for a while, again filtered, washed with water, and taken up in dilute sodium carbonate solution. Any undissolved neutral product was filtered out and the solution acidified, thus giving (0·5 g.) more of the glutaric acid. The glutaric acid is soluble in acetone and acetic acid, sparingly so in alcohol or ethyl acetate and insoluble in water, petrol, or benzene. It gave an insoluble barium salt in cold (Found : C—68·92% ; H—6·96 % ; Eq—201 ; $C_{23}H_{28}O_6$ requires C=69·00% ; H=7·00% ; Eq—200. Found : Ba = 25·2% ; $C_{23}H_{26}O_6Ba$ requires Ba=25·6%).

The glutaric acid, by warming with concentrated sulphuric acid, gave a mixture mainly of the dilactone m.p. 184° C. and 6-methyl-coumarin-4-acetic acid, with a small amount of the monolactonic acid m.p. 205° C. Similarly the monolactonic acid (2 g.) by hydrolysis and ethylation with 25% sodium hydroxide (25 c.c.) and diethyl sulphate (5 c.c.) gave the glutaric acid m.p. 219° C. (decomp.).

The *diethyl ester* could also be prepared from the glutaric acid by alcohol and sulphuric acid (Found : C—70·8% ; H—7·8% ; $C_{27}H_{36}O_6$ requires C=71·00% ; H=7·90%).

The *dimethyl ester* crystallised from methyl alcohol in colourless flat needles, m.p. 105° C. (Found : C=69·90% ; H—7·4% ; $C_{25}H_{32}O_6$ requires C=70·10% ; H=7·47%).

Unlike the glutaconic acids, these glutaric esters do not condense with aromatic aldehydes. On condensing the dimethyl ester (1 mol.) with dimethyl oxalate (3 mols.) in the presence of sodium methoxide, according to Dieckemann's method,[7] most of it was recovered unchanged. Very small amount of a substance melting above 160° C. was however obtained as a more soluble fraction, which on further examination appeared to be a complex mixture.

The *anhydride* was prepared from the glutaric acid by the action of acetic anhydride or by thermal decomposition at 220° C. temperature. It crystallised from benzene in colourless flat rods, m.p. 189° C. It is insoluble even in caustic alkalies, and does not give any colouration with ferric chloride in cold alcoholic solution : (Found : C—72·08% ; H=6·73% ; $C_{23}H_{26}O_5$ requires C=72·25% ; H=6·8%).

The *acid-anilide* of the glutaric acid was prepared from the above anhydride and aniline in benzene solution, and crystallised from 80% alcohol in colourless silky needles m.p. 193° C. (decomp.) (Found : C=73·00% ; H=6·7% ; $C_{29}H_{33}O_5N$ requires C=73·24% ; H=6·9%).

The *anil* is formed by the action of aniline on the glutaric acid at a temperature of 220° C. or by the decomposition of the above acid anilide at its melting point. It was crystallised from alcohol in colourless rectangular prisms m.p. 216° C. It is insoluble in even boiling caustic alkalies and does not give any colouration with ferric chloride (Found : C = 75·91% ; H = 6·62% ; $C_{29}H_{31}O_4N$ requires C = 76·15% ; H = 6·78%).

ββ'-(22'-dimethoxy-55'-dimethyl-diphenyl)-glutaric acid.—This dimethoxy acid corresponding to the diethoxy, could not be had either in the condensation of *p*-cresol-methylether with acetone-dicarboxylic acid, or by condensing the β-(2-methoxy-5-methyl-phenyl)-glutaconic acid with *p*-cresol-methyl-ether (see below). This glutaric acid was however prepared from the above dilactone m.p. 184° C. by hydrolysis and methylation. The compound crystallised from alcohol in colourless rectangular prisms, m.p. 192° C. (decomp.). It gave an insoluble barium salt in cold (Found : C – 67·62% ; H = 6·38% ; Eq. = 188 ; $C_{21}H_{24}O_6$ requires C – 67·74% ; H – 6·45% ; Eq. – 186).

Condensation of p-cresol-methylether *with* acetone-dicarboxylic acid (improved method).—Finely powdered citric acid (200 g.) was mixed with concentrated sulphuric acid (200 c.c.) and fuming sulphuric acid (20% SO_3, 120 c.c.) was later added. The mixture was shaken well and heated on a water bath at 60° C. temperature till the evolution of carbon monoxide stopped and a clear orange solution was obtained. This was cooled in a freezing mixture, *p*-cresol-methylether (80 c.c.) gradually added with shaking and the reaction mixture, on standing for 3 hours, poured over 800 g. of crushed ice. The thus separated sticky solid became crystalline on keeping overnight, was then filtered, and treated with dilute sodium carbonate solution to remove any unchanged *p*-cresol-methylether when on acidification 52 g. of mixed acids were obtained. This was treated with boiling 10% acetic acid (1000 c.c.) and filtered, when the filtrate on cooling deposited crystals of the β-(2-methoxy-5-methyl-phenyl)-glutaconic acid. The glutaconic acid crystallised from water in colourless rectangular plates m.p. 167° C. (decomp.). Gogte and Limaye[8] give the m.p. 169° C. (decomp.).

22'-dimethoxy-55'-dimethyl-chalkone-a-acetic acid.—The portion insoluble in acetic acid in above, was crystallised from alcohol. This was dissolved in least amount of boiling 10% sodium carbonate solution, which on cooling deposited the sodium salt of the chalkone-acetic acid as colourless leaflets. These were collected at the pump under strong suction, acidified with hydrochloric acid, and the chalkone-acetic acid thus obtained was recrystallised from alcohol in colourless parallelogramic plates, m.p. 252° C. yield 12 g. The acid is a remarkably stable compound being unchanged even on fusion

with solid potash. It is soluble in acetone, sparingly so in acetic acid or alcohol, and insoluble in other organic solvents. It gave an insoluble barium salt in cold. Its ethyl ester did not condense with aromatic aldehydes (Found : C—71·00% ; H=6·13% ; Eq. 346 ; $C_{21}H_{22}O_5$ requires C=71·2%; H=6·2% ; Eq.—354. Found : Ba=15·6% ; $(C_{21}H_{21}O_5)_2Ba$ requires Ba—16·25%).

*The *ethyl-ester* crystallised from 70% methyl alcohol in parallelogramic plates m.p. 122° C. (Found : C=72·1% ; H—6·7% ; $C_{23}H_{26}O_5$ requires C=72·24% ; H=6·8%).

*The *semicarbazone* was obtained by refluxing the alcoholic solution of the chalkone-acetic acid with semicarbazide hydrochloride and sodium acetate for 4 hours. When recrystallised from alcohol, it melted at 277° C. (decomp.) (Found : C=64·00% ; H=5·92% ; $C_{22}H_{25}O_5N_3$ requires C=64·24% ; H= 6·08%).

*The *semicarbazone of the ethyl-ester* crystallised from alcohol in colourless needles m.p. 219° C. (decomp.) (Found : C—65·38% ; H—6·44% ; $C_{24}H_{29}O_5N_3$ requires C—65·6% ; H=6·6%).

Synthesis of the chalkone-acetic acid m.p. 252° C. from β-(2-methoxy-5-methyl-phenyl)-glutaconic acid and p-cresol-methylether.— The glutaconic acid (5 g.) was finely powdered and dissolved in previously ice-cooled dilute—4 vols. acid : 1 vol. water—sulphuric acid (40 c.c.). p-cresol-methylether (10 c.c.) was then gradually added with shaking and the whole allowed to stand overnight at the room temperature. On pouring the reaction mixture on 100 g. of powdered ice, a partly sticky mass, becoming granular on keeping for few hours, separated. It was filtered, washed, treated with 100 c.c. of boiling 10% aqueous acetic acid, and filtered. The chalkone-acetic acid insoluble in acetic acid, was purified as above. Yield 1·5 g. The acetic acid filtrates on cooling gave the 6-methyl-coumarin-4-acetic acid.

Action of sulphuric acid on the chalkone-acetic acid m.p. 252° C.—On keeping a solution of the chalkone-acetic acid (2 g.) in 80% sulphuric acid (15 c.c.) overnight at room temperature, the indone-acetic acid (1·1 g.) melting at 218° C. (decomp.), was obtained, and was found identical with that described by Gogte and Limaye.[3] Action of concentrated sulphuric acid (10 c.c.) on the glutaconic acid (2 g.) at 60° C. for 1 hour, gave on pouring the reaction mixture in water and filtering, 1·4 g. of a precipitate. It was washed with boiling water, treated with boiling dilute sodium carbonate solution and

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* These three compounds had been described in the M.Sc. Thesis of the author, but the melting points of the latter two were given as 272°C. and 212°C., and their method of preparation was also different.

separated into 0.6 g. of the above indone-acetic acid and 0.8 g. of a neutral compound. This was recrystallised from 40 c.c. of alcohol in parallelogramic plates m.p. 214° C. (Found: C—74.87%; H—5.82%; $C_{21}H_{20}O_4$ requires C—75.00%; H—5.95%).

The *semicarbazone of the neutral* melted at 263° C. (decomp.) (Found: C=61.65%; H=5.6%; disemicarbazone, $C_{23}H_{26}O_4N_6$ requires C=61.30%; H—5.78%; monosemicarbazone $C_{22}H_{23}O_4N_3$ requires C—67.17%; H=5.85%).

Benzylidene derivative of the neutral.—The neutral compound was dissolved in alcohol and refluxed with benzaldehyde and alcoholic potash for one hour. Alcohol was evaporated, the residue rubbed with water to remove the alkali, and the unreacted benzaldehyde removed with steam. On crystallisation from alcohol the benzylidene compound melted at 174° C. (Found: C—78.7%; H—5.41%; $C_{28}H_{24}O_4$ requires C=79.24%; H—5.66%).

The ethyl-ester of the above chalkone-acetic acid m.p. 252° C., when treated with concentrated sulphuric acid at 50–60° C. produced the ethyl ester m.p. 158°C. of the above indone-acetic acid m.p. 218° C., mainly and a small amount of the indone-acetic acid itself, but the above neutral compound m.p. 214° C. could not be detected. Concentrated nitric acid at room temperature transformed the chalkone-acetic acid into the indone-acetic acid, and hence its oxidation with nitric acid yielded the phthalic anhydride m.p. 186° C. identical with that obtained by Limaye and Gogte[8] (*Ref.* M.Sc. Thesis of the author).

The author wishes to thank Dr. P. C. Guha, D.Sc., for the encouragement he has given and the facilities provided in carrying out the present investigation; and also to Prof. D. B. Limaye, Poona, for the helpful suggestions he gave during the progress of this work, as well as Part I of this series.

NOTE :—With regard to Part I of this series (This Journal Vol. I, No. 1, pp. 48–60) the author wishes to state the following :—The words "while working at the Ranade Institute for his M.Sc. Thesis" should be inserted after the sentence "in extending Limaye and Bhave's method" (p. 48, line 2 from bottom). The new method of synthesis of β-aryl glutaconic acids described therein, was discovered in the case of the glutaconic acids from *p*- and *m*-cresol-methylethers, during the last term of the stay of the author at the Ranade Institute, Poona, and the extension of this new method of synthesis to the glutaconic acids of the naphthol series, was carried out at the Indian Institute of Science, Bangalore.

ON THE p-POTENCY OF G $(p^\mu - 1, r)$.

By Hansraj Gupta,

Government College, Hoshiarpur.

§1.   In this paper, I prove two generalisations of Wilson's Theorem and some potency properties of G $(p^\mu - 1, r)$.   I make a free use of the results proved in an earlier paper.[3]   G (n, r) denotes the sum of the products of the first n natural numbers taken r at a time.   I denote by $\underset{a < \cdot n}{\mathrm{G}} (a, r)$ the sum of the products taken r at a time of all numbers less than and prime to n. p stands in general for an odd prime unless stated otherwise.

§2.   *Theorem.*   G $[np - 1, m\,(p-1)] \equiv (-1)^m \binom{n}{m} \pmod{p}$.

I have shown[3] that

$$\mathrm{G}\,(p+j, r) \equiv \mathrm{G}\,(j, r) \pmod{p}\,;\ 0 < r \leqslant p - 2\,; \qquad \cdots \qquad \cdots \qquad (3\cdot3)[4]$$

and

$$\qquad\qquad = \mathrm{G}\,(j, r) - \mathrm{G}\,(j, r - p + 1) \pmod{p}\,;\ r > p - 1. \cdots \quad (3\cdot31)[4]$$

Applying these reductions k times, we get

$$\mathrm{G}\,[np - 1,\, m\,(p-1)] \equiv \sum_{i=0}^{m} \left[(-1)^i \binom{k}{i} \mathrm{G}\,\{(n-k)\,p - 1,\, (m-i)\,(p-1)\} \right]$$

$$\pmod{p}.$$

Let $k = n - 1$, then

$$\mathrm{G}\,[np - 1,\, m\,(p-1)] \equiv (-1)^{m-1} \binom{n-1}{m-1} \mathrm{G}\,(p-1, p-1) + (-1)^m$$

$$\mathrm{G}\,(p-1, 0) \binom{n-1}{m} \pmod{p},$$

$$= (-1)^m \binom{n}{m} \pmod{p}, \text{ since}^3\ \mathrm{G}\,(p-1, p-1)$$

$$\equiv -1 \pmod{p}.$$

In fact, if $r \not\equiv 0 \pmod{p-1}$,
then G $(np - 1, r) \equiv 0 \pmod{p}$.
For, let $r \equiv s \pmod{p-1}$, $1 \leqslant s \leqslant p - 2$.

Then reducing $(n-1)$ times as before, we get

$$G (np-1,\ r) \equiv (-1)^m \binom{n-1}{m} G (p-1, s) \pmod{p}, \text{ where } m = \left[\frac{r}{p-1}\right]$$

$$\equiv 0 \pmod{p}, \text{ since}[3]\ G (p-1, s) \equiv 0 \pmod{p}.$$

In particular $G (p^u-1, r) \equiv 0 \pmod{p}$, except when $r=0$ or $\phi (p^u)$.

§3. *Theorem.* $G [p^u-1, \phi (p^u)] \equiv \pm 1 \pmod{p^u}, u \geqslant 1, p \geqslant 2.$

It is easily shown that

$$G [p^u-1, \phi(p^u)] = \underset{a<\cdot p^u}{G} [a, \phi (p^u)] + \sum_{r=1}^{p^{u-1}-1} \{p^r G (p^{u-1}-1, r).$$
$$\underset{a<\cdot p^u}{G} [a, \phi (p^u)-r]\},$$

when $p > 3$, we have[2] $p^r G (p^{u-1}-1, r) \equiv 0 \pmod{p^u}$.

Hence, $G [p^u-1, \phi (p^u)] \equiv \underset{a<\cdot p^u}{\text{II}} (a) \equiv -1 \pmod{p^u}, p > 3,$

when $p = 2$, and $u = 1, 2$; $G [p^u -1, \phi (p^u)] \equiv -1 \pmod{p^u}$,

when $p = 2$, and $u \geqslant 3$,

$$p^r G (p^{u-1}-1, r) \equiv 0 \pmod{p^{u-1}}, r = 1, 2 ;$$
$$\equiv 0 \pmod{p^u}, \quad r \geqslant 3.$$

Moreover $\underset{a<\cdot p^u}{G} [a, \phi (p^u)-r] \equiv \begin{bmatrix} \phi (p^u) \\ r \end{bmatrix} \pmod{2}$,

$$\equiv 0 \pmod{2}, \text{ since } u \geqslant 3, r = 1, 2.$$

Hence $G [p^u-1, \phi (p^u)] \equiv \underset{<\cdot p^u}{\text{II}} (a) \equiv 1 \pmod{p^u}, p = 2, u \geqslant 3.$

Thus $G [p^u-1, \phi (p^u)] \equiv \underset{a<\cdot p^u}{\text{II}} (a), p = 2, u \geqslant 1; \pmod{p^u}.$

In general $G (p^u-1, r) \equiv \underset{a<\cdot p^u}{G} (a, r) \pmod{p^u} p \geqslant 3 ;$

and $\equiv \underset{a<\cdot p^u}{G} (a, r) \pmod{p^{u-1}}, p = 2.$

In particular when $r > \phi (p^u)$,

$$G (p^u-1, r) \equiv 0 \left(\text{mod. } p^{u-\left[\frac{2}{p}\right]}\right),\ p \geqslant 2.$$

§4. Let $\omega (r)$ denote the p-potency of $G (p^u-1, r)$, $p \geqslant 3, r \geqslant 1, u \geqslant 1$. Then we have[3]

$$r G (p^u-1, r) = \binom{p^u}{r+1} + \sum_{k=1}^{r-1} \left[G (p^u-1, k) \binom{p^u-k}{r-k+1}\right]. \qquad .. \quad (3\cdot2)[4]$$

If in this equation r is given in succession the values $1, 2, 3\ldots\ldots, p-2$, we easily prove that $\omega (r) \geqslant u, 1 \leqslant r \leqslant p-2.$ $\qquad .. \qquad .. \qquad$ (A)

Putting $p-1$ for r, we get $\omega (p-1) = u-1.$

Again $G\ (p''-1, r) = \overset{r}{\underset{m=1}{\Sigma}}\left[\underset{m}{f}(r)\left(\genfrac{}{}{0pt}{}{p''}{2r-m+1}\right)\right],$ .. .. .. $(1 \cdot 3)^4$

Where the f's are positive integers[1] given recursively by

$$\underset{m}{f}(r) = (2r-m)\,[\underset{m}{f}(r-1) + \underset{m-1}{f}(r-1)],\ \underset{0}{f}(r) = 0.$$

when $r \geqslant p$, it is easily proved that

$$\underset{m}{f}(r) \equiv 0\ (\text{mod.}\ p),\ m = 1, 2, 3, \ldots, p-4 + \left[\frac{2r+4}{p+1}\right];$$

also $\underset{m}{f}(r) \equiv 0\ (\text{mod.}\ p),\ m = r, r-1, r-2, \ldots, r+1 - \left[\frac{r-1}{p-1}\right].$

Let β be the p-potency of the most potent of the integers

$$r+1+\left[\frac{r-1}{p-1}\right], r+2+\left[\frac{r-1}{p-1}\right], r+3+\left[\frac{r-1}{p-1}\right], \ldots, (2r+4)-p$$

$$-\left[\frac{2r+1}{p+1}\right], r \geqslant p.$$

Then $\omega\ (r) \geqslant u - \beta,\ r \geqslant p.$ .. .. (B)

Moreover, $2\ G\ (p''-1, 2i+1) \equiv \left(\genfrac{}{}{0pt}{}{p''-2i-1}{1}\right)p''\ G\ (p''-1, 2i),\ i \geqslant 1,$

(mod. $p \geqslant 2u$). .. .. $(3 \cdot 1)^4$

Hence $\omega\ (2i+1) \geqslant 2u-\beta+\gamma,$ .. .. (C)

where γ is the p-potency of $(2i+1)$, and $p^\beta \leqslant 4i < p^{\beta+1}$.

In particular $\omega\ (p\delta) \geqslant 2u,\ 1 \leqslant \delta.$ .. .. (D)

§5.   *Theorem.* $\omega\ [\phi\ (p^\lambda)] = u - \lambda,\ 0 < \lambda \leqslant u,\ p \geqslant 2.$

We have[3] $G\ (p''-1, r) = \overset{r}{\underset{m=1}{\Sigma}}\left[\underset{m}{f}(r)\cdot\left(\genfrac{}{}{0pt}{}{p''}{2r-m+1}\right)\right],$ .. .. $(1 \cdot 3)^4$

where the f's are positive integers independent of p and u.   Let $r=\phi\ (p^\lambda)$, then the p-potency of $G\ (p''-1, \phi\ (p^\lambda)$ will be definitely known if from the terms on the right-hand side of $(1 \cdot 3)^4$, we can single out one with a potency less than that of any and every other of the terms.

I proceed to show that such a term is

$$\underset{m}{f}(r)\left(\genfrac{}{}{0pt}{}{p''}{p^\lambda}\right),\ r=\phi\ (p^\lambda),\ m=p^\lambda-2p^{\lambda-1}+1.$$

Since $G\ [p^\lambda-1, \phi\ (p^\lambda)]$ is 0-potent in p,

therefore $\underset{p^\lambda-2p^{\lambda-1}+1}{f}(r)$ is 0-potent in p; $r=\phi\ (p^\lambda)$.

Moreover,

$\left(\genfrac{}{}{0pt}{}{p''}{p^\lambda}\right)$ is less potent in p than every other member of $\left(\genfrac{}{}{0pt}{}{p''}{t}\right)$,

where $1 + \phi\ (p^\lambda) \leqslant t \leqslant 2\phi\ (p^\lambda)$.

A7   F

Hence $\omega\,[\phi\,(p^\lambda)] = u - \lambda,\ 0 < \lambda \leqslant u.$ .. .. .. (E)

In view of the results of §3, we now get

$$\underset{a < \cdot p^u}{G}\,[a, \phi\,(p^\lambda)] \equiv 0 \ (\text{mod. } p \leqslant u - \lambda),\ p \geqslant 2,\ 1 \leqslant \lambda \leqslant u. \qquad \text{..} \qquad \text{(F)}$$

In other words $G\,[p^u - 1, \phi\,(p^\lambda)]$ and $\underset{a < \cdot p^u}{G}\,[a, \phi\,(p^\lambda)],\ p \geqslant 2,\ 1 \leqslant \lambda < u,$

are equi-potent in p.

§6.　*Theorem.*　$\omega\,(p^u - 2) \geqslant \dfrac{p^u - 1}{p - 1} - 2u + \chi,\ u \geqslant 1,$

where $\chi = 1$, 2 or 3 according as $p = 2$, 3, or > 3.

We have $G\,(p^u - 1,\ p^u - 2) = (p^u - 1)\,! \ \underset{a=1}{\overset{p^u - 1}{\Sigma}}\ \dfrac{1}{a}.$

$$= (p^u - 1)\,! \left[\underset{a < \cdot p^u}{\Sigma}\,\dfrac{1}{a} + \dfrac{1}{p}\,\underset{a < \cdot p^{u-1}}{\Sigma}\,\dfrac{1}{a} + \ldots + \dfrac{1}{p^{u-1}}\,\underset{a < \cdot p}{\Sigma}\,\dfrac{1}{a} \right].$$

The theorem follows immediately from Wolstenholme's Theorem,[5] *viz.,*

$$\underset{a < \cdot p^k}{\Sigma}\,\dfrac{1}{a} \equiv 0 \ (\text{mod. } p^{2k-l}),$$

where $l = 0$, 1, 2, according as $p = > 3$, 3, or 2.

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3.　. . . . . . "Congruence Properties of G-Functions," *Proc. Edinburgh Math. Soc.* (2), 1935, 4, 61-66.

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IRIDIUM ISOTOPES AND THEIR NUCLEAR SPINS.

BY B. VENKATESACHAR

AND

L. SIBAIYA.

(*Department of Physics, University of Mysore, Bangalore.*)

IRIDIUM is practically the only element whose isotopic constitution has not so far been revealed by the mass-spectrograph. The present authors have arrived at the isotopic constitution of platinum from a study of the hyperfine structure of its arc lines.[1] Almost simultaneously Dempster[2] has published the isotopic analysis of this element with the aid of his mass-spectrograph. He has more recently studied the isotopes of palladium and gold,[3] whose hyperfine structure data obtained by one of us is under publication. Of the four elements, *viz.*, Pd, Ir, Pt and Au, mentioned by Aston[4] as having withstood attempts to arrive at their isotopic constitution by the use of the mass-spectrograph, iridium alone remained without any information in this respect. A study of the hyperfine structure of the arc lines of iridium was undertaken with the view of determining its isotopes as well as their nuclear spins.

The hollow cathode used in this investigation is exactly similar to the one used previously ;[5] instead of the platinum foil, an iridium sheet (supplied by Messrs. Johnson Matthey & Co., Ltd., London) was inserted in the hollow cathode. A discharge current of about 200 mA. at 1000 v. brings up the iridium arc lines arising from transitions to the low-lying levels with enough intensity to enable a study of their hyperfine structure. The advantage of the particular type of hollow cathode employed here lies in the fact that only a few lines corresponding to transitions to ground and near low-lying levels are excited without any reversal. Fig. 1 shows that in platinum as well as in iridium the lines for which the hyperfine structure study has been possible are those arising from transitions to a few of the deepest levels only. It is a matter of great advantage in hyperfine structure work to obtain such significant lines of an element intensely without any complications of self-reversal.

[1] Venkatesachar and Sibaiya, *Nature*, 1935, **136**, 65.
[2] Dempster, *Nature*, 1935, **135**, 993.
[3] Dempster, *Nature*, 1935, **136**, 65.
[4] Aston, *Proc. Roy. Soc.*, 1935, **149**, 404.
[5] Venkatesachar and Sibaiya, *Proc. Ind. Acad. Sci.*, 1935, **1**, 955-960.
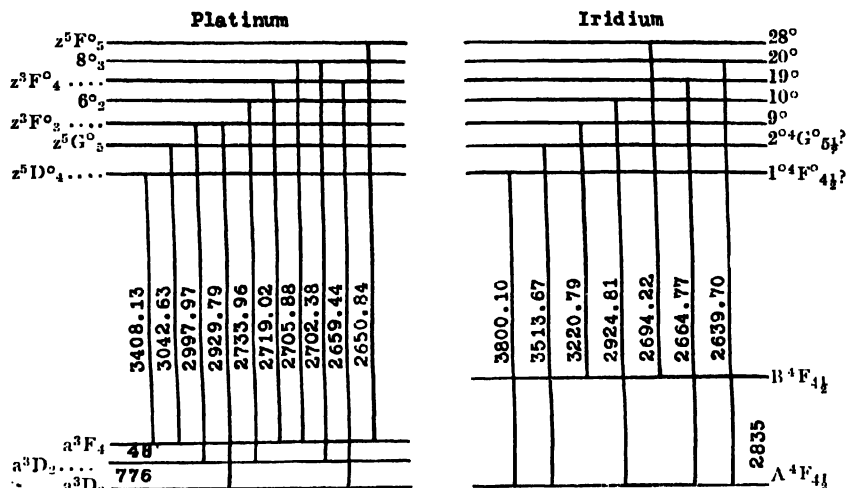
FIG. 1.   Arc lines of platinum and iridium analysed.   (Not to Scale.)

The spin separations even in the deepest levels of iridium are so small that many of the arc lines just appeal widened and exhibit no structure. The three arc lines λλ 3800·10, 3513·67 and 2924·81 Å have a common lower level in the ground term A $^4F_{4\frac{1}{2}}$ according to Albertson.[6]   These three lines exhibit similar structure ; but the components are so very close together that accurate measurements have been possible only in the intense line λ 3513·67 Å.   The similarity in structure and the approximate equality of the component separations suggest that the structure in these lines must be attributed to a spin splitting of the ground term A $^4F_{4\frac{1}{2}}$ with the upper levels unsplit. λ 3513·67 Å exhibits the following structure in cm.$^{-1}$ and the visual estimates of relative intensity are included in brackets :

IrI λ 3513·67 Å (5d^{8}6s $^4F_{4\frac{1}{2}}$ – 5d^{8}6p $^4G_{5\frac{1}{2}}$)

dv in cm.$^{-1}$ (Int.)	Remarks
+0·072 (7)	This component appears as a continuous patch between +0·072 and 0·000, indicating the presence of an expected satellite at +0·033 (9), vide Pl VIII.
0·000 (22)	Broad.
–0·073 (13)	
–0·115 (9)	

6 Albertson, *Phys. Rev.*, 1932, **42**, 443.

Judging from the known isotopic constitution of elements with odd atomic numbers, iridium can have either *one* or *two* odd isotopes. If one assumes the existence of only one isotope, the chemical atomic weight 193·1 suggests that the isotope is Ir 193. No value of nuclear spin for Ir 193 even with the lower and upper levels both split can give the observed structure ; this fact has been established by the method of Fisher and Goudsmit employed for explaining the structure of insufficiently resolved lines. Another alternative is that iridium should consist of two isotopes Ir 193 and Ir 195, because the chemical atomic weight is 193·1. Examining all the known odd isotopes of elements, it is found that 191 and 193 are the only two odd mass numbers missing in the neighbourhood of iridium ; 195 has been previously shown to exist in platinum.[1,5] It appears further that the isotopes of an element with odd atomic number usually have no isobars in appreciable quantity. Since the isotope of mass 195 exists to an extent of about 30% in platinum,[7] the existence of Ir 195 in any quantity is very improbable. Remembering that no element with odd atomic number exhibits more than two odd isotopes, one need only consider 191 and 193 as the isotopes of iridium. Examining the data on the atomic weight determinations of iridium, it is found that there is great divergence in the values given by various investigators ranging from 192·59 to 193·40. In these circumstances much importance cannot be attached to the value of the atomic weight 193·1. In many cases where the mass-spectrograph has decided against the chemical atomic weight as in Tb, Tm, Au, Ta, etc., the chemical atomic weight has been in excess of the true value. The observed structure of the lines is accounted for uniquely by assuming the existence of the two isotopes 191 and 193 with the respective nuclear moments of $\frac{1}{2}\frac{h}{2\pi}$ and $\frac{3}{2}\frac{h}{2\pi}$. The

$\Delta i = 0$			$\Delta i = \pm 1$				
		i			i		i
Cl	35, 37	$\frac{5}{2}$					
Cu	63, 65	$\frac{3}{2}$	Hg	199	$\frac{1}{2}$	Hg	201	$\frac{3}{2}$
Ga	69, 71	$\frac{3}{2}$					
Br	79, 81	$\frac{3}{2}$	Rb	87	$\frac{3}{2}$	Rb	85	$\frac{5}{2}$
Cd	111, 113	$\frac{1}{2}$					
Sn	117, 119	$\frac{1}{2}$	Sb	121	$\frac{5}{2}$	Sb	123	$\frac{7}{2}$?
Ba	135, 137	$\frac{3}{2}$					
Eu	151, 153	$\frac{5}{2}$	Xe	129	$\frac{1}{2}$	Xe	131	$\frac{3}{2}$
Re	185, 187	$\frac{5}{2}$					
Tl	203, 205	$\frac{1}{2}$	Ir	191	$\frac{1}{2}$	Ir	193	$\frac{3}{2}$

[7] Venkatesachar and Sibaiya, *Proc. Ind. Acad. Sci.*, 1935, **2**, 101–103.

addition of two neutrons to the nucleus of the odd isotope of lower mass gives rise to an odd isotope of heavier mass with a change in nuclear spin of either 0 or ± 1; the change is 0 when the two added neutrons have opposite spin moments and is ± 1 when their spins have the same sign. Tamm and Altschuler[8] also consider that sometimes two neutrons do not form a closed shell with zero spin, but add up to give a spin of 1. The above table contains all the known cases where the nuclear spin change between two odd isotopes of an element is either 0 or ± 1.
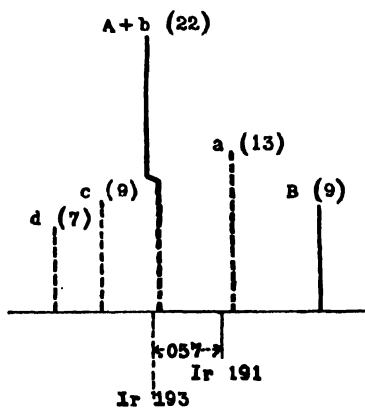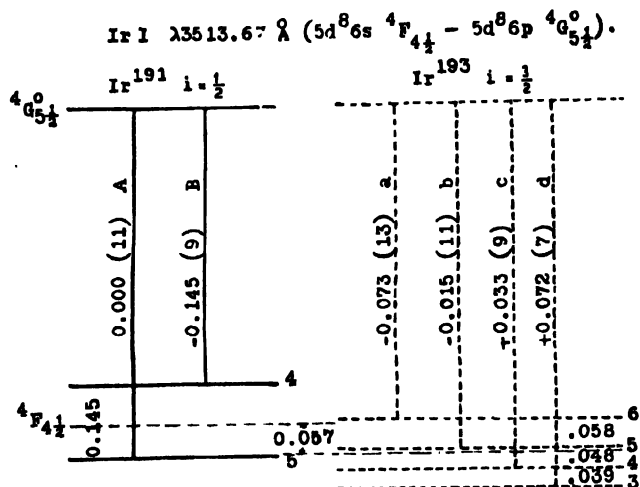
Fig. 2.

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[8] Tamm and Altschuler, "*Ac*", *U.R.S.S.*, 1934, 1, 455.

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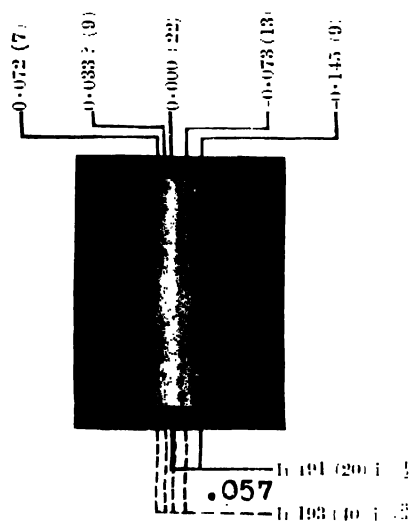
The observed structure of λ 3513·67 Å is explained on the basis of two isotopes 191 and 193 with nuclear spins of $\frac{1}{2}$ and $\frac{3}{2}$ respectively in Fig. 2. The eye-estimates of the intensities of the components is consistent with theoretical expectations and give for the relative abundance of the isotopes 191 and 193 a ratio approximately equal to 1 : 2. The correct ratio however must await a microphotometric study of the structure pattern. The above estimate gives an atomic weight of 192·4, which is perhaps a little too low. There is no doubt however that the accepted chemical atomic weight is too high, and that the correct atomic weight of iridium must lie between 192 and 193. An accurate estimate of the relative abundance of the isotopes is further impeded by the uncertainty of the J-value of the ground term ; Meggers and Laporte give it as $2\frac{1}{2}$ while Albertson gives it as $4\frac{1}{2}$. The ground term exhibits an isotope displacement of 0·057 cm. [1] with the heavier isotope lying deeper as in the cases of copper and thallium.[9] The existence of this isotope shift favours the suggestion that the ground term of iridium is a $^4F_{4\frac{1}{2}}$ term as suggested by Albertson arising from an electronic configuration $5s^2 5p^6 5d^8 6s$ in preference to a $^2D_{5,2}$ term from $5s^2 5p^6 5d^9$ as given by Meggers and Laporte[10]. It is for this reason that the J-value of the ground term has been here assumed as $4\frac{1}{2}$ in calculations of intensity, intervals and isotope shift. The incompletely resolved patterns of a few other IrI lines 3220·79, 2694·22, 2664·77 and 2639·70 support the conclusions obtained above regarding the isotopes of iridium and their nuclear spin. The fine structure levels in $B^4F_{4\frac{1}{2}}$ of Albertson are regular in Ir 191 and inverted in Ir 193, while in $A^4F_{4\frac{1}{2}}$ the reverse is the case. The ratio of the nuclear magnetic moments of the two isotopes is therefore about −1·0.

Summary.

The hyperfine structure patterns of some of the significant arc lines of iridium have been photographed using as source a hollow cathode tube already described in a paper by the authors on the isotopic constitution of platinum. An examination of the hyperfine structure data of the iridium lines leads to the result that it consists of two isotopes with nuclear spins $\frac{1}{2}\frac{h}{2\pi}$ and $\frac{3}{2}\frac{h}{2\pi}$. A consideration of known facts regarding the occurrence of isotopes of different mass numbers in the various elements has led to the inference that the mass numbers of the two iridium isotopes are 191 and 193 with a relative abundance of nearly 1 : 2, the isotope with the higher mass number having the higher nuclear spin.

[9] Venkatesachar and Sibaiya, *Proc Ind. Acad. Sci.*, 1934, **1**, 13.
[10] Meggers and Laporte, *Phys. Rev.*, 1926, **28**, 660.

PROCEEDINGS

OF

THE INDIAN ACADEMY OF SCIENCES

CONTENTS

SECTION B

INDIAN WATER-MOULDS—I.

By H. Chaudhuri

AND

P. L. Kochhar.

(*From the Department of Botany, Panjab University.*)

Introduction.

THE majority of the water-moulds belong to *Saprolegniaceæ*, having some 80 species in 14 genera known at present. They live mostly as saprophytes on dead and decaying animal and vegetable matters submerged in water. Some also live as parasites. Most of these water-moulds have been recorded in Europe and America and have been described by de Bary (1881, 1888), Massee (1891), Humphrey (1893), Klebs (1899), Kauffman (1908), Petersen (1910), Von Minden (1912, 1916), Pieters (1915), Coker (1923), Coker and Braxton (1926), Harvey (1925), Kanouse (1927), Fitzpatric (1930) and others. The list of British species has been considerably added to by Ramsbottom (1904-1906), Barnes and Melville (1932), Cook and Forbes (1933) and Forbes (1935). Besides the work of Butler (1907, 1911) describing a few forms, there has been very little done in India. Recently a *Myzocitium* has been described by Chaudhuri (1931).

Apart from systematic studies, the growth of the water-moulds under different cultural conditions has been studied, and inoculation experiments have been made with a pathogenic form. Twenty species, *viz.*, 19 species belonging to 5 genera of *Saprolegniaceæ* and one *chytrid* belonging to *Rhizidiaceæ* have been recorded and described. This paper forms the first of a series of papers it is intended to publish on water-moulds.

Method and Technique.

As the water-moulds reproduce freely by means of zoospores, samples of water were collected in tubes from different localities. A boiled house-fly was placed for 24 hours in each of these tubes after which the fly was washed in several changes of sterilised water and transferred to a Petri dish, containing sterilised distilled water. In a day or two, the fly which is infected by the zoospores, becomes covered by fungal hyphæ which radiate out of the body of the fly. It is then transferred to another Petri dish and fresh transfers are always made every third day. It has been observed that usually a

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number of species occur in a sample of water, and the fly gets infected by more than one species of mould. Hence it is necessary to isolate them and grow in pure culture. This was done by picking up, under a binocular microscope, a single hypha bearing a zoosporangium by means of needles and placing that in a Petri dish containing sterilised distilled water, after it had been washed again in several changes of water. In a day or two fungal hyphæ grow around the bits of sterilised egg-yolks placed in those dishes which become infected by the zoospores liberated by the zoosporangium.

Many a time certain seeds and pieces of fruits have been used as baits.

Often cultures get contaminated by bacteria unless the water is slightly acidulated. If an infected fly bearing mature zoosporangia from a bacteria-contaminated dish be washed in several changes of acidulated water, and another fly-bait is gently placed at the opposite end of the same dish in slightly acidulated water and left for about 2 hours, then from the latter a bacteria-free culture may be obtained.

The life-history of the moulds has been studied in the living condition by mounting in water and staining with neutral red. Escape of zoospores and the different stages in the development of the spores could thus be easily followed.

In making permanent mounts, the following procedure was followed. The material was fixed in a solution of formalin (10 c.c.), glacial acetic acid (5 c.c.), and water (85 c.c.) for 24 hours. After washing the material in several changes of water, this was put in 10% glycerine containing ·01 % erythrosine in a watch glass and allowed to dry slowly for two days. When the glycerine thickened, the material was immersed at once in absolute alcohol and then through the usual grades of alcohol and xylol was finally mounted in canada balsam after spreading out the material with a pair of needles. These preparations suffered no shrinkage at all and the stain also did not fade. Mounts were also made directly from the material in thickened glycerine, in either pure glycerine or in glycerine jelly ringed with asphaltam. These latter mounts very soon lost the brilliancy of the stains and after a few months the stain almost faded.

General Characters.

These fungi consist of cœnocytic threads attached to rhizoid-like system. Hyphæ either branched or more or less simple; sub-hyaline. Septa appear only in connection with reproductive bodies. Asexual reproduction by means of zoospores. Zoosporangia formed at the tips of the hyphæ; of various shape and size; renewed by internal proliferation as in *Saprolegnia* or by cymose branching from below the older ones as in *Achlya* or by both methods

as in *Isoachlya*. Zoospores liberated singly or in groups. Dark coloured resting bodies (gemmæ or chlamydospores or conidia) formed sometimes. These either grow into new hyphæ or produce zoospores. Sexual reproduction by means of oogonia and antheridia. Oogonia formed at the tips of main hyphæ or on short lateral branches or sometimes intercalary, singly or in chains. Eggs one to many in an oogonium. Antheridia short or long, simple or branched and either originating from the same thread on which the oogonia are borne (Androgynous), or from other thread (Diclinous). Fertilisation tubes present or may be absent.

Descriptions of Species and Their Cultural Characters.

Genus *Saprolegnia* Nees v. Esenbeck, 1823, p. 513.

Hyphæ slender, more or less branched, primary sporangia terminal, long clavate, often slender fusiform, polymorphic in old cultures, secondary ones produced by proliferations through the older ones; zoospores liberated singly and swim away separately.

1. *S. parasitica* Coker. Sap. 1923, p. 57, Pl. II.

Growth rather delicate, dense, short, reaching $0 \cdot 9$ cm. on egg-yellow in distilled water; chlamydospores abundant, size and shape variable, often in chains of 2–4, last in the series often spherical or globular. Hyphæ $24 \cdot 5$–$36\,\mu$. Zoosporangia variable, often bent and irregular, cylindrical to long clavate with rounded tips, 45–60×280 $340\,\mu$; occasionally $560\,\mu$ long, proliferating internally through the older ones. . Zoospores $9 \cdot 5$–$10 \cdot 8\,\mu$.

On boiled house-fly in tap water and distilled water—delicate growth, short hyphæ, abundant zoosporangia. On boiled egg-yellow in distilled water—zoosporangia and also chlamydospores formed.

2. *S. parasitica* Coker, Var. *Kochhari*, n. var.

Pl. V. Figs. 1–6.

Parasitic on *Belone Cancinula* and other fishes. Abundant hyphæ covering the dead fish. In laboratory *Labio rohita* (8″ long) infected by means of zoospores, but *Ophiocephalous punctatus*, though injured at the mouth from which hyphæ grew out, recovered and lived for three months.

Dense long hyphæ, zoosporangia usually very long and stouter; bigger class *S. parasitica*. 50–75×400–$600\,\mu$ long. Zoospores usually not liberated but germinate inside the zoosporangium when growing on fish.

If zoosporangium is put in distilled water, zoospores are liberated in the usual manner. Microtome sections of the infected fish showed the presence of the fungus throughout the body of the host.

Coll. : S. R. Kashyap, Hiran Minar, Sheikhupura, Punjab.

Genus *Aplanes* de Bary, 1888, p. 613.

Hyphæ slender, very minute, sparingly branched or simple, zoosporangia very rare, almost absent.

3. *A. sp.* Pl. I. Figs. 20–29.

Growth delicate but extensive, reaching upto 2 cm. on coagulated egg-yellow. Hyphæ slender, occasionally branched, $9 \cdot 2 - 15 \cdot 3 \mu$ in thickness. Zoosporangia very rare, when present cylindrical to long clavate, $28–30 \cdot 3 \mu$; renewed by internal proliferation, one zoosporangium observed with cymose branching. Chlamydospores abundant, very variable in size and shape, elliptical to globular or irregularly cylindrical, terminal or intercalary, in chains or single, sexuality not observed.

Growth in culture.— On house-fly in tap water—only vegetative growth with a few gemmæ, zoosporangia rare. On egg-yellow in distilled water and in boiled corn grain—abundant chlamydospores formed. Old cultures on egg-yellow when transferred to $0 \cdot 1\%$ leucine solution showed no change in two weeks.

Collected from Lahore, Amritsar and Gujranwala.

Genus *Isoachlya*, Kauffman, 1921, p. 231.

Hyphæ rather stout or slender. Zoosporangia terminal, variable in shape and size, secondary ones arising either by cymose arrangement, as in *Achlya*, or by internal proliferation as in *Saprolegnia*. Zoospores escaping and swarming separately. Oogonia without antheridia in most cases.

4. *I. monilifera* (de Bary) Kauffman, *Amer. Journ. Bot.*, 1921, **8,** 231.

Pl. VI. Figs. 1–9.

Vegetative growth rather extensive and stout, reaching $1 \cdot 5$ cm. on egg-yellow in distilled water. Hyphæ $14–27 \mu$ occasionally upto 33μ. Sporangia not abundant, usually cylindrical to clavate, proliferating internally. Zoospores $11 \cdot 4 - 12 \cdot 2 \mu$. Chlamydospores variable, terminal or intercalary, usually in chains, the terminal one being globular or spherical. Oogonia very abundant throughout the culture, usually in chains, most of them breaking up easily from the hyphæ and from each other on maturity, typically spherical with a long neck, wall thin, diameter $49–91 \mu$. Eggs 1–30, mostly 4–12; $21–25 \mu$ centric. Antheridia absent.

Growth in culture.—House-fly in distilled water–growth rich, zoosporangia developed with zoospores liberated, oogonia not so plentiful. Egg-yellow in distilled water and in boiled corn grain—growth luxuriant, plenty of oogonia of normal shape with mature eggs, no antheridia. On house-fly in $0 \cdot 1\%$

solution of potassium phosphate—growth not so thick, hyphæ scattered with very few oogonia, zoosporangia normally developed.

Collected from Batala and Shalamar.

This species differs from Coker's description of *I. monilifera* in having constantly long-necked oogonia and larger number of eggs in the oogonium.

<center>Genus *Protoachlya* Coker. Sap., 1923, p. 90.</center>

Hyphæ rather delicate. Zoosporangia cylindrical to flask-shaped with rounded tips, thickest near the apex. Zoospores diplanetic, some remaining attached in an irregular clump at the tip of the zoosporangium, other swimming away separately.

5. *P. paradoxa* n. comb. (Coker).

<center>Pl. VI. Figs. 10-25.</center>

Achlya paradoxa Coker, *Mycologia*, 1914, **6**, 285, Pl. 146.

Isoachlya paradoxa (Coker) Kauff., *Amer. Journ. Bot.*, 1921, **8**, 231.

Growth open, delicate, long reaching 1·75 cm. on egg-yellow in distilled water. Hyphæ 12·3 18·6 μ, occasionally thicker upto 28 μ. Zoosporangia very abundant, 27·6–33·8 μ in diameter, tips rounded or occasionally broad, secondary ones arising usually by cymose branching beneath the older ones. Zoospores diplanetic, formed in several rows, showing great variation in behaviour, some moving away separately after emergence, others forming an irregular group at the tip. Chlamydospores terminal, rarely intercalary, mostly in chains, oblong to clavate, sometimes cylindrical, zoospores oblong to oval within the zoosporangium and spherical outside, their long axes parallel to the wall of the zoosporangium during escape, 12·5–13·2 μ. Oogonia on short lateral branches, rarely terminal or intercalary, spherical, 33–60 μ in diameter, mostly 43–47 μ, those near the substratum with many eggs and those outward with few, usually 1–2. Eggs spherical 18–24 μ. Antheridia present on all oogonia mostly diclinous, occasionally androgynous, sometimes more than one to an oogonium.

Growth in culture.— On house-fly and on egg-white in distilled water—growth extensive, delicate, hyphæ thick near the base. Many sporangia with *Achlya*-like proliferation. On egg-yellow in distilled water—as above. Abundant chlamydospores formed. Oogonia formed only in old cultures, some disorganising. On corn grain in distilled water—growth vigorous, many zoosporangia and chlamydospores, no oogonia. On house-fly in 0·1% solution of leucine—abundant gemmæ and many oogonia with ripe eggs. On house-fly in 0·1% solution of potassium phosphate—growth fairly strong, zoosporangia and chlamydospores numerous, zoospores liberated normally.

Collected from Sheikhupura and Atari.

It may be noted that it has smaller-sized eggs than those described by Coker.

Genus *Achlya* Nees v. Esenbeck, 1923, p. 514.

Zoospores from a common mouth, collecting in a group at the tip of the zoosporangia ; zoosporangia thicker than the vegetative hyphæ, zoospores not in a single row.

6. *A. americana* Humphrey.

PI. VII. Figs. 1–11.

Growth dense, rather stout, upto 2 cm. on egg-yellow in distilled water. Hyphæ stout giving out slender branches, upto 78 μ near the base, branches usually 27·6–48 μ, finer branches as small as 12·3 μ, zoosporangia long, cylindrical, with wavy margin, 24·6–36·9 μ in thickness, proliferating frequently from below the older ones. Zoospores liberated in a group, 8·3–9·1 μ. Chlamydospores not so frequent, formed by the segmentation of hyphæ into elongated pieces, usually of the same thickness as the zoosporangium. Oogonia plentiful borne on short lateral stalk, rarely in chains, spherical, occasionally flask-shaped, wall pitted, 40·1–68·7 μ in diameter, mostly 46 μ. Oogonial stalk varying from less than to more than twice the diameter of oogonium. Eggs 2–6 sometimes upto 13–15, eggs spherical 21·5–23·8 μ, sub-centric to eccentric. Antheridia mostly androgynous, occasionally diclinous, simple.

Growth in culture.—On house-fly in tap water—vegetative growth fairly dense, normal sporangia ; chlamydospores and oogonia nil. On egg-white in distilled water—growth delicate, open. On egg-yellow in distilled water—growth close, strong, hyphæ branched, few chlamydospores, oogonia plentiful, all maturing and forming ripe eggs. On corn grain in distilled water—growth extensive, oogonia few, maturing late. A culture on house-fly transferred to 0·1% solution of leucine formed oogonia with ripe eggs in 5 days.

Collected from Shalamar and Lahore.

It differs from Coker's description in the smaller size of its zoospores and oogonia and in the smaller number of eggs in the normal oogonia. Forbes has also described this species recently from Manchester, and her description shows this species to be closely allied to *A. debaryana* but the latter differs from this in having unpitted oogonial walls and some diclinous antheridia, while the former has pitted oogonial walls and consistently androgynous antheridia.

7. *A. proliferoides* Coker.

P. VII. Figs. 12-19.

Growth open, stout, long, upto 2 cm. on egg-yellow in distilled water. Hyphæ more or less branched, usually wavy, tapering, varying greatly in size, 135 μ near the base, ultimate branches as fine as 15·7 μ, tips withering. Zoosporangia abundant, long, narrowly cylindrical, sometimes bent, 30·7-38 × 210-490 μ, occasionally 50 μ thick, often opening by one or more lateral pores. Zoospores 10·5-11·2 μ, coming out in one continuous line and forming an irregular group at the mouth. Oogonia abundant, spherical, rarely irregular, 45-47 μ in diameter, on short lateral stalks, which vary from as long as, to 1½ times as long as the diameter of the oogonium ; oogonial wall thin, pitted. Eggs 1-8, spherical 21-30 μ, averaging 25 μ. Antheridia diclinous, branching a single one sending branches to 2 or more oogonia.

Growth in culture. On house-fly and on boiled egg-white in distilled water — only vegetative growth, zoospores all liberated at maturity. On egg-yellow in distilled water—vegetative growth strong and healthy, few oogonia developed, remaining immature for a long time, all with antheridial branches. Oogonia abundant on corn grain. On corn grain in distilled water— vegetative growth extensive, zoosporangia and zoospores well developed ; many mature oogonia with ripe eggs ; all with antheridia. A culture on house-fly transferred to 0·1% solution of leucine produced oogonia in 5 days ; more than 75% with ripe eggs, antheridia on relatively few oogonia.

Collected from Gujranwala and Lahore.

It will be noted that the size of the eggs is bigger than those described by Coker for the new species.

8. *A. flagellata* Coker. Sap. 1923, p. 116.

Pl. VIII. Figs. 1-8.

Growth somewhat dense, reaching about 2 cm. on egg-yellow in distilled water. Hyphæ branching, tapering gradually, 30-39 μ thick, main hyphæ at the base upto 120 μ, often dying back at the tip. Zoosporangia plentiful, sub-cylindrical to cylindrical, very variable in length, often long and thick, 49-54 × 306-709 μ, slightly bent and occasionally with more than one lateral opening. Zoospores coming out in a mass and gradually falling to the bottom, 9·8-11 μ thick. Gemmæ not so frequent. Oogonia fairly plentiful, usually on lateral stalks, which may be twice as long as the diameter of the oogonium, spherical, 41-78 μ in diameter, mostly 52-58 μ, wall pitted, not so thick, about 1·3-1·6 μ. Eggs 1-9, mostly 3-6, spherical, eccentric, 21·3-29 μ in diameter. Antheridia both diclinous and androgynous, more often former, branching attached by means of feet to the oogonial wall.

Growth in culture.—On house-fly in distilled water—vegetative growth short but dense ; zoosporangia and a few chlamydospores formed, no oogonia· On egg-white in distilled water—growth open, hyphæ long, with very few, if any, branches ; zoosporangia with zoospores, which are easily liberated. On egg-yellow in distilled water—growth strong and dense ; zoosporangia abundant, zoospores germinating within the zoosporangium or outside ; chlamydospores abundant, oogonia formed late. On a fly in 0.1% solution of Potassium phosphate—few hyphæ coming out and growing to a considerable length, stout. On a fly in 0.1% solution of Potassium nitrate—growth fair, only vegetative, few sporangia, no oogonia or chlamydospores.

Collected from Amritsar, Lahore and Gujranwala.

10.   *A. imperfecta* Coker. Sap. 1923, p. 118.

<div align="center">Pl. VIII. Figs. 9-20.</div>

Growth dense, not so delicate, reaching 1.75 cm. on egg-yellow. Hyphæ near the substratum stout, giving out delicate branches, branches dying back at the tips ; 9.2 $30.4\,\mu$, mostly 12.3–$15.3\,\mu$. Zoosporangia abundant, sub-cylindrical, somewhat irregular in outline, renewed in a cymose manner or formed anew below the older ones, 30.3–39.9×520–$1205\,\mu$. Zoospores 10-$10.8\,\mu$ thick, dark, spherical. Chlamydospores formed by the segmentation of hyphæ, mostly sub-cylindrical and in chains, rarely ovate with lateral branches, often getting loosened and separated from each other and the threads. Oogonia plentiful, spherical or sub-spherical, occasionally irregular in outline and with papillæ on short lateral stalks which are less than, to more than $1\frac{1}{2}$ times the diameter of the oogonium ; with or without a neck, 55.2–$67.3\,\mu$ in diameter, averaging $59\,\mu$, wall unpitted. Eggs 2–8 in an oogonium, mostly 3–5, eccentric, 21–$28.3\,\mu$ mostly $21.3\,\mu$, spherical or sometimes elliptical. Antheridia both androgynous and diclinous, more or less branched, a single one sending branches to more than one oogonium.

Growth in culture.—On house-fly in distilled water—hyphæ stout, vegetative growth dense, covering the fly ; chlamydospores few, zoospores developed normally and all liberated. On egg-white and boiled corn grain and egg-yellow—oogonia developed in a week's time, zoospores formed, also a few chlamydospores. A culture of egg-yellow in distilled water transferred to 0.1% solution of leucine produced abundant oogonia in 3 to 4 days. Some of these disorganised, zoosporangia short. On house-fly in 0.1% solution of Potassium nitrate—only vegetative growth seen. On house-fly in 0.05% solution of Potassium chloride—only few threads came out and these died in a week.

Collected from Lahore and Amritsar.

10.   *A. Kashyapia*, n. sp.

Pl. IX. Figs. 1-10.

Growth rather open, hyphæ stout near the substratum, giving out slender branches, 12·3–18·4μ, occasionally as wide as 30 μ and as fine as 9·2 μ. Zoosporangia plentiful, elongated, cylindrical or sub-cylindrical, more or less smooth, secondary ones quite common and produced as in other forms of this genus, very variable in size 32–45×252 873·7μ, occasionally as long as 1 mm., those on finer branches as small as 15·4 × 175 μ. Chlamydospores formed usually by the segmentation of hyphæ and therefore in chains and cylindrical to sub-cylindrical, rarely otherwise, not getting loosened and separated from each other. Zoospores 9·8–10·5μ. Oogonia not so frequent, when present scattered in groups of three or four, on short lateral stalks, dark coloured, neck short or absent, spherical, occasionally flask-shaped. Wall moderately thick, 2·8 3·1 μ, unpitted. Diameter 58·2 76·3μ. Eggs 3–8 in number, 24·6–27·8μ, spherical, sub-centric or centric. Antheridia not on all oogonia, diclinous, branching, a single one sending branches to 2 or more.

Growth in culture.--On fly in distilled water--vegetative growth rich ; zoosporangia formed and zoospores liberated. Growth poor on egg-white. On egg-yellow in distilled water—vegetative growth very luxuriant, upto 1·5 cm., oogonia plenty, remaining immature for a long time, mature ones degenerating; zoospores not developed in all zoosporangia, liberated early. On corn grain in distilled water—vegetative growth dense and long, upto 2 cm. ; oogonia formed but not very abundant, eggs mature ; chlamydospores seen. A culture on house-fly when transferred to 0·1% solution of leucine produced abundant oogonia in 2 days, eggs ripen. On corn meal agar and corn grain (boiled) in 0·1% solution of Potassium phosphate—growth moderate, few sporangia and gemmæ. On house-fly in 0·1% solution of Potassium nitrate --good growth 1 cm. ; zoosporangia and chlamydospores developed.

Collected from Lahore and Khasa (near Amritsar).

A. Kashyapia approaches *A. prolifera* in having diclinous antheridia but differs from the latter in the unpitted character of the oogonial wall and in its larger eggs. It also resembles *A. imperfecta* except in the size of the oogonia and eggs and in the centric or sub-centric character of its eggs. It may be a synthetic species.

11.   *A. Klebsiana* Pieters, *Bot. Gaz.*, **60**, 486.

Pl. IX. Figs. 11-17.

Moderately stout, growth on egg-yellow reaching 1·75 cm. Hyphæ 30-54μ, main threads upto 84 μ at the base, tapering gradually, branched. Zoosporangia 36-49 ×429-502μ, occasionally 71·8 μ thick, those at the ends

of fine branches as small as $15 \times 270 \mu$. Secondary ones quite common, arising
cymosely. Zoospores $10 \cdot 7$- $11 \cdot 3 \mu$. Chlamydospores formed by the segmenta-
tion of hyphæ, rod-like. Oogonia plentiful, borne on short lateral stalks
which are from less than, to $2\frac{1}{2}$ times the diameter of the oogonium, spherical
or sub-spherical, with or without a neck, 39–60μ, mostly 47–55μ. Oogonial
wall thin, $1 \cdot 5$–$2 \cdot 3 \mu$. Eggs 1-6, usually 3-4, 20–$26 \cdot 2 \mu$, mostly $23 \cdot 8 \mu$;
spherical or irregularly flattened by pressure, centric. Antheridial branches
slender, always diclinous, androgynous very rare.

Growth in culture.—On house-fly in distilled water—vegetative growth
good, zoosporangia forming zoospores, some liberating, others retaining them,
oogonia after about 8 -9 days. On egg-yellow in distilled water—vegetative
growth extensive, reaching $1 \cdot 75$ cm., oogonia abundant, all with antheridia.
On egg-white in distilled water—vegetative growth delicate, zoosporangia
not developing zoospores ; oogonia only few. A culture on fly in $0 \cdot 1\%$ solu-
tion of leucine produced abundant oogonia, only 50% forming ripe eggs, no
chlamydospores. On house-fly in $0 \cdot 1\%$ solution of Potassium phosphate—
only vegetative growth, zoospores not developed, no chlamydospores.

Collected from Shalamar, Mughalpura and Lahore.

It agrees with Forbes' description of the species.

12. *A. conspicua* Coker, Sap. 1923, p. 131.

Pl. X. Figs. 1-8.

Growth dense, reaching $1 \cdot 5$ cm. on egg-yellow in distilled water. Main
hyphæ long and stout, upto 145μ thick near the base, fine branches as small
as $24 \cdot 6 \mu$; zoosporangia abundant, broadly cylindrical, 43–$52 \cdot 2 \times 247$- 558μ.
Secondary ones abundant, produced in groups or borne in a zigzag manner
at long intervals. Zoospores $8 \cdot 9$–$9 \cdot 9 \mu$. Oogonia somewhat plentiful, on
short lateral stalks, which are at least as long as the diameter of the oogonium,
spherical or oval, occasionally cylindrical and notched, $58 \cdot 3$–$64 \cdot 5 \mu$, wall
yellowish, not thick, slightly pitted. Eggs 3-7, spherical, $24 \cdot 6$–$30 \cdot 7 \mu$.
Antheridia androgynous, branched.

Growth in culture.—On house-fly in distilled water—vegetative growth
fair, rather stout ; zoosporangia abundant, short, almost oblong. On
egg-yellow in distilled water—growth dense, chlamydospores nil. Oogonia
with ripe eggs formed after 20 days.

Collected from Amritsar, Atari and Lahore.

13. *A. dubia* Coker, Sap. 1923, p. 135. Pl. XLIX.

Pl. X. Figs. 9-14.

Growth rather dense, $1 \cdot 5$ cm. on egg-yellow. Main threads stout, more
or less branched, $21 \cdot 3$–$40 \cdot 7 \mu$, thick, tapering gradually. Zoosporangia

abundant, terminal, long cylindrical or somewhat clavate, $30 \cdot 7 - 42 \cdot 9 \mu$, often thinner, those on finer branches as short as $23 \times 270 \mu$. Zoospores $10-11 \cdot 3 \mu$. Oogonia on short lateral branches, which vary in length from less than, to $1\frac{1}{2}$ times the diameter of the oogonia, $61 \cdot 4 - 70 \cdot 5 \mu$, wall thin, not pitted. Eggs 2 9, $24 \cdot 6 - 30 \cdot 8 \mu$, mostly $27 \cdot 6 \mu$, many without antheridia, others with 2 or more. Antheridia diclinous, attached by feet.

Growth in culture.—On house-fly in distilled water—vegetative growth fair, about 1 cm., open, hyphæ stout, zoosporangia plentiful, no oogonia. On egg-yellow in distilled water—as above, oogonia formed only in old cultures after one month.

Collected from Lahore, Amritsar and Gujranwala.

14. *A. dubia* Coker, var. *pigmenta*, n. var.

Pl. XI. Figs. 1–12.

Growth slightly dense, hyphæ stout, reaching 1 cm. on egg-yellow. Hyphæ $27 \cdot 6 - 36 \cdot 8 \mu$, branched, straight. Zoosporangia thicker, $36-67 \times 370-720 \mu$, long cylindrical, outlines regular and straight. Secondary ones arising by cymose branching from below the primary ones. Chlamydospores abundant, produced in groups, of various shapes. Zoospores coming out occasionally in groups and collecting at the tip, mostly retained within the zoosporangium and germinating there. Oogonia plentiful, on short lateral stalks, which are upto as long as the diameter of the oogonium ; diameter $39 \cdot 2 - 62 \mu$, wall smooth, thin, yellowish or dark. Eggs 2–9, usually 3–4 ; $21 \cdot 4 - 24 \cdot 5 \mu$ in diameter. Antheridia diclinous or androgynous, mostly former, branched, attached by feet to the oogonial wall.

Growth in culture.—On house-fly in distilled water—vegetative growth dense, zoosporangia abundant, only few chlamydospores, no oogonia. Chlamydospores abundant on boiled corn grain. On egg-yellow in distilled water —vegetative growth fair, chlamydospores abundant, oogonia after a week, some without antheridia, zoospores liberated or retained in some. A culture on corn grain transferred to $0 \cdot 1\%$ solution of leucine produced abundant oogonia ; antheridia on all.

Collected from Lahore and Gujranwala.

A. dubia pigmenta differs from *A. dubia* in its small-sized oogonial stalks and smaller eggs. It is characterised by its chlamydospores, coloured oogonial wall and constantly occurring diclinous antheridia.

15. *A. deBaryana* Humphrey.

Pl. XI. Figs. 9–31.

Growth very dense ; $1 \cdot 75$ cm. on egg-yellow. Hyphæ strong, $19-26 \mu$ thick, more or less branched, or ending in zoosporangia, which are somewhat

elongated or occasionally clavate, $27 \cdot 6$–$30 \cdot 7 \mu$ thick ; secondary ones not so common. Zoospores large, $12 \cdot 1$–$14 \cdot 6 \mu$. Chlamydospores very abundant, of various shapes mostly in chains or branched, each opening by one or more lateral pores. Oogonia abundant, borne on short lateral stalks, or very often intercalary, single or in chains, varying much in form, normally spherical to sub-spherical, very often of abnormal shape, cylindrical or oval, occasionally double, rarely with one or more papillæ. 47–$61 \cdot 4 \mu$ in diameter. Wall thin and smooth. Eggs 2-many, spherical centric, $21 \cdot 6$–$30 \cdot 7 \mu$; antheridia not always present, diclinous or androgynous, mostly latter.

Growth in culture.—On house-fly and on egg-white in distilled water—growth rather open, zoosporangia and chlamydospores abundant ; oogonia formed in cultures, after 15 days. On egg-yellow in distilled water—growth dense and long ; zoosporangia not so abundant ; chlamydospores plentiful, of various shapes ; oogonia developed luxuriantly in 2–3 days ; many abnormal oogonia occur, occasionally double and produced in old zoosporangia, cylindrical. A culture on house-fly transferred to $0 \cdot 1\%$ solution of leucine produced very few oogonia, variously shaped ; few chlamydospores here and there. On egg-yellow in $0 \cdot 1\%$ solution of Potassium phosphate—growth fair, rather open, few chlamydospores ; oogonia rare. On fly in $0 \cdot 1\%$ solution of Potassium nitrate- growth delicate, open, very few hyphæ.

Collected from Amritsar.

This species agrees with Forbes' description of the species fundamentally.

16. *A. aplanes* Maurizio. *Flora*, 1894, **79**, 135, Pl. 4–5.

Pl. XII. Figs. 1–10.

Growth dense, stout, $1 \cdot 2$ cm. on egg-yellow. Hyphæ delicate, fine, scarcely branched $12 \cdot 3$–$27 \cdot 6 \mu$ thick, wavy. Zoosporangia thicker, elongated or narrow clavate, $31 \cdot 4$–40×222·504μ, occasionally as fine as $19 \cdot 1 \mu$. Chlamydospores abundant, cut off from below the zoosporangia by transverse septa, therefore in chains, cylindrical. Oogonia abundant borne on short lateral stalks, which vary from as long as, to more than twice as long as the diameter of the oogonium, $45 \cdot 3$–$67 \cdot 5 \mu$ in diameter, spherical or slightly ovate, wall smooth, thick and yellowish. Eggs 1–6, spherical, $24 \cdot 5$–$27 \cdot 6 \mu$, eccentric. Antheridia not always present, mostly diclinous, branched.

Growth in culture.—On house-fly in distilled water—zoosporangia and chlamydospores only. A few oogonia on egg-white. On egg-yellow in distilled water—dense vegetative growth, $1 \cdot 5$ cm., oogonia plentiful with ripe eggs, many eggs degenerating, zoospores often retained. On a fly in

0·1% solution of Potassium phosphate—only isolated hyphæ here and there, scarcely branched, some bearing zoosporangia at their tips. On housefly in 0·1% solution of Potassium chloride- -no growth.

Collected from Amritsar and Gujranwala.

Forms without Oogonia.

17. *A. sp.* No. 1.

Pl. XII. Figs. 12-14.

Growth open, 1 cm. on egg-yellow. Hyphæ delicate, more or less branching, upto 150 μ near the sub-stratum, ultimate branches as fine as 9·5 μ. Zoosporangia abundant, or narrowly cylindrical, 39·9 × 227 μ, with a long apical papilla ; secondary ones rare, no chlamydospores. Sexuality not observed.

Growth in culture.—A study of this was made in all the cultures previously used, but no sexual reproduction was observed.

Collected from Lahore.

18. *A. sp.* No. 2.

Pl. XII. Figs. 15-16.

Growth dense near the base, open outwards, 1·5 cm. on egg-yellow in distilled water. Hyphæ moderately stout, slightly branched, upto 83 μ near the base, falling to 30 μ outwards. Zoosporangia broadly cylindrical or narrowly oblong, 30-38 × 402-568 μ. Chlamydospores abundant formed by the segmentation of hyphæ.

Growth in culture.—As in *A. sp.* no. 1.

Collected from Lahore.

19. *A. sp.* No. 3.

Pl. XII. Figs. 17-19.

Growth rather dense, delicate upto 2 cm. on egg-yellow. Hyphæ 30-51 μ ; zoosporangia long clavate to cylindrical with pointed ends, 40-46 × 70-140 μ. Chlamydospores abundant, formed by the segmentation of hyphæ, therefore in chains, those lower in the series usually elliptical, the terminal one cylindrical ; zoospores germinating within the zoosporangia.

Growth in culture.—As in *A. sp.* no. 1. No sexuality induced in any culture medium.

20. *A. sp.* No. 4.

Pl. XII. Figs. 20-23.

Growth open, moderately stout, short, 1 cm. on egg-yellow. Hyphæ strong, 55·8 -70 μ at the base, upto 33·5 μ outwards, profusely branched. Zoosporangia more or less oblong or occasionally cylindrical ; secondary

ones few, 1-2 at a point, $39 \cdot 9 - 48 \times 350 - 440 \mu$, cylindrical ones as long as 1050μ. Chlamydospores abundant, terminal or intercalary, single or in chains, usually short, sub-globose, ovoid or flask-shaped, often elliptic to rectangular. Zoosporangia and chlamydospores sometimes with apical papillæ. Zoospores coming out in mass, spherical, $9 \cdot 8 - 10 \cdot 2 \mu$.

Growth in culture.—Usual cultural study of this form has been made, but all the media employed in other forms failed to induce sexuality ; only on corn grain in distilled water, chlamydospores were more abundant than in others. Zoospores always liberated.

Collected from Amritsar.

Family *Rhizidiaceae.* Genus *Rhizidiomyces* Zopf (1884 : 188).

21. *R. apophysatus* Zopf, *Nova Acta Cad, Leop.*, 1884, **47,** 188, Pl. 20, Figs. 1-7.

Pl. XII. Figs. 24 29.

Sporangia spherical or sub-spherical, varying greatly in size, from 9μ to 22μ, seated on the surface of the oogonia of *A. Klebsiana* and sending fine tube-like bodies into the interior of the host. Spores $3 \cdot 5 - 3 \cdot 7 \mu$ thick. Disorganises the eggs, which break into pieces, and prevents their further development.

Found parasitic on the oogonia of a culture of *A. Klebsiana*, developed from sample No. 66.

Summary and List of Species Noted.

An examination has been made of the waters from various pools and ditches of the Lahore district of the Punjab and 20 species of water-moulds, most of which have not been noted before in this country and some new forms, have been recorded. These have been grown in various culture media and their cultural characteristics studied. Full diagnoses of the species have been given and differences, if any, from the older descriptions have also been noted. All the types described in this paper are fully illustrated. Inoculation experiments with a parasitic form—*Saprolegnia parasitica* var. *Kocchari*, n. var.—have been made. The following types have been described :—

Saprolegnia parasitica ; S. parasitica var. *Kocchari ; Aplanes* sp. ; *Isoachlya monilifera ; Protoachlya paradoxa ; Achlya americana ; A. proliferoides ; A. flagellata ; A. imperfecta ; A. Kashyapia* n. sp. ; *A. Klebsiana ; A. conspicua ; A. dubia ; A. dubia* var. *pigmenta ; A. deBaryana ; A. aplanes ; A. sp.* Nos. 1 to 4 (without oogonia) and *Rhizidiomyces apophysatus.*

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EXPLANATION OF PLATES.

(*N.B.*—All figures reduced to 7/16 in reproduction.)

PLATE VI.

Figs. 1–6.—*Saprolegnia parasitica*, var. *Kocchari*.

Figs. 1–2.—Different stages in zoospore formation. $\times$ 140.

Figs. 3–4. Germination of zoospores inside zoosporangium. $\times$ 140.

Fig. 5.—*Belone cancinula* naturally infected. Reduced ¾.

Fig. 6. Photos of 3 other fishes, artificially infected. Reduced ½.

Figs. 7–19. *Saprolegnia parasitica*.

Figs. 7, 8, 9 & 10. Various forms of zoosporangia showing internal proliferation. $\times$ 140.

Fig. 11. Zoospores. $\times$ 610.

Fig. 12.—A part of zoosporangia showing internal proliferation. $\times$ 372.

Figs. 13, 14, 15, 16, 17 & 18.—Chlamydospores. $\times$ 140.

Fig. 19. Zoosporangia proliferating. $\times$ 140.

Figs. 20–29.—*Aplanes* Sp.

Figs. 20–21.—Zoosporangia, (1) showing both internal and lateral proliferation; (2) showing internal proliferation. $\times$ 140.

Fig. 22.—Zoosporangia. $\times$ 610.

Fig. 23.—Intercalary chlamydospore. $\times$ 140.

Fig. 24.—Intercalary and terminal chlamydospores. $\times$ 140.

Figs. 25, 26, 27 & 28.—Various forms of chlamydospores. $\times$ 140.

Fig. 29.—Chlamydospores metamorphosed into zoosporangium. $\times$ 282.

PLATE VI.

Figs. 1–9.—*Isoachlya monilifera*.

Fig. 1.—Internally proliferating zoosporangium with zoospores. $\times$ 372.

Fig.   2. Chlamydospores, one with zoospores, some germinating within. $\times$ 372.
Figs.   3-4. Zoosporangia (3) $\times$ 83; (4) $\times$ 140.
Fig.   5. Chlamydospore. $\times$ 83.
Fig.   6.—Habit of oogonia with ripe eggs. $\times$ 372.
Fig.   7. An oogonium on a zoosporangium. $\times$ 140.
Fig.   8. A double oogonium with small eggs. $\times$ 372.
Fig.   9. An oogonium with ripe eggs. $\times$ 610.
Figs. 10 25.—*Protoachlya paradoxa.*
Fig.   10. Habit of zoosporangia. $\times$ 140.
Fig.   11. Zoosporangium liberating zoospores. $\times$ 140.
Fig.   12. A part of zoosporangium showing elliptical zoospores. $\times$ 610.
Fig.   13.—Zoospores, one germinating. $\times$ 610.
Fig.   14. Part of zoosporangium, zoospores spherical. $\times$ 610.
Figs. 15 16.- Habit of chlamydospores. $\times$ 372.
Fig.   17. Oogonium. $\times$ 610.
Fig.   18. Oogonia with antheridia. $\times$ 372.
Figs. 19 24. Chlamydospores of various types. $\times$ 140.
Fig.   25. Chlamydospore metamorphosed to zoosporangium. $\times$ 610.

PLATE VII.

Figs.   1-11. *Achlya americana.*
Fig.   1.—Oogonium with diclinous antheridia. $\times$ 645.
Figs.   2-3. Habit of zoosporangia. $\times$ 140.
Fig.   4. Zoospores. $\times$ 610.
Fig.   5.—Habit of chlamydospores. $\times$ 140.
Fig.   6. Habit of oogonia. $\times$ 140.
Fig.   7. Oogonium with androgynous antheridia. $\times$ 645.
Fig.   8. Cylindrical oogonium with ripe eggs. $\times$ 280.
Figs.   9 10. Oogonia with zoosporangia. $\times$ 140.
Fig.   11. Large oogonium with ripe eggs. $\times$ 390.
Figs. 12-19. —.*Achlya proliferoides.*
Figs. 12 13. Habit of zoosporangia. $\times$ 145.
Figs. 14 15.—Habit of chlamydospore. $\times$ 145.
Fig.   16.- Habit of oogonia. $\times$ 145.
Fig.   17.—Oogonia with diclinous antheridia. $\times$ 390.
Fig.   18. Oogonia with zoosporangium. $\times$ 145.
Fig.   19. Young oogonium with antheridium. $\times$ 645.

PLATE VIII.

Figs.   1-8. *Achlya flagellata.*
Figs.   1 2. Habit of zoosporangium. $\times$ 146.
Fig.   3. Zoospores. $\times$ 610.
Fig.   4.—Zoosporangia with lateral openings. $\times$ 83.
Fig.   5.—Habit of oogonia. $\times$ 140.
Fig.   6.—Oogonia on zoosporangia. $\times$ 83.
Fig.   7. Mature oogonium with ripe eggs. $\times$ 372.
Fig.   8.- Young oogonium with antheridia. $\times$ 372.
Figs.   9 20. *Achlya imperfecta.*
Fig.   9.—Photograph of a culture on egg yolk.
Figs. 10–11.—Habit of zoosporangia. $\times$ 145.
Fig.   12. Zoospores. $\times$ 610.
Figs. 13-14. Chlamydospores (4) $\times$ 88; (5) $\times$ 145.

Fig.   15.—Habit of oogonia.   × 140.
Figs. 16-17.—Peculiarly notched double oogonia.   × 390.
Fig.   18.—Chlamydospores.   × 390.
Figs. 19-20.—Oogonia with antheridia.   × 390.

PLATE IX.

Figs. 1-10.—*Achlya Kashyapia.* n. sp.
Figs. 1-3. Habit of zoosporangia too with lateral openings.   × 140.
Fig.   4.—Habit of oogonia.   × 140.
Fig.   5.— Oogonia with diclinous antheridia.   × 372.
Fig.   6.—Chlamydospores.   × 83.
Fig.   7.—Double oogonium, the central egg shaped as the oogonium.   × 610.
Figs. 8-10.— Oogonia with antheridia.   (3, 5) × 610; (4) × 372.
Figs. 11-17.—*Achlya Klebsiana.*
Figs. 11-13. Habit of zoosporangia.   × 145.
Fig.   14.— Zoospores.   × 610.
Fig.   15.—Habit of oogonia.   × 145.
Figs. 16-17.—Oogonia with antheridia.   × 390.

PLATE X.

Figs. 1-8.—*Achlya conspicua.*
Fig.   1. —Dichtyuchus like zoosporangia.   × 83.
Fig.   2. Habit of oogonia.   × 88.
Fig.   3.—Zoosporangia with peculiar oogonium.   × 145.
Figs. 4-5.—Oogonia of abnormal shapes.   × 390.
Fig.   6.—Notched oogonium with antheridia.   × 390.
Fig.   7.—Zoosporangia showing branching.   × 140.
Fig.   8. -Typical habit of zoosporangia.   × 140.
Figs. 9-14. *Achlya dubia.*
Figs. 9-10.— Habit of zoosporangia.   × 140.
Fig.   11.—Zoospore formation.   × 610.
Fig.   12. Habit of oogonia.   × 140.
Fig.   13. Oogonium with diclinous antheridia.   × 372.
Fig.   14.—An oogonium with mature eggs.   × 610.

PLATE XI.

Figs. 1-12.—*Achlya dubia* var. *pigmenta* n. var.
Figs. 1-2. Normal habit of zoosporangia.   × 140.
Fig.   3.—Zoosporangium.   × 372.
Fig.   4.- Zoospores.   × 610.
Fig.   5.—Habit of oogonia.   × 610.
Fig.   6.—Two oogonia in chains.   × 610.
Fig.   7.—A large oogonium with ripe eggs.   × 610.
Fig.   8. -Oogonium with diclinous antheridia.   × 610.
Figs. 9-31.—*Achlya de Baryana.*
Fig.   9.—Zoosporangia.   × 140.
Figs. 10-11 & 13-16.—Chlamydospores of various types.   × 140.
Fig.   12.—Two types of oogonia borne on a hypha.   × 83.
Fig.   17.—Intercalary oogonia in chains.   × 372.
Fig.   18. -An oogonium with a long papilla.   × 610.
Figs. 19-21.—Abnormal types of oogonia (11, 12) × 372;   (13)   × 610.

Bl A

Fig. 22.—An oval oogonium with an antheridium. × 610.
Fig. 23.—A cylindrical oogonium. × 372.
Fig. 24.—Oogonium in an old zoosporangium. × 140.
Figs. 25–26.—Abnormal oogonia. × 372.
Fig. 27.—Oogonium at the tip of chlamydospores. × 372.
Fig. 28.—Triangular oogonium with ripe eggs. × 372.
Fig. 29.—An abnormal oogonium. × 140.
Figs. 30–31.—Oogonia with zoosporangia. × 140.

Plate XII.

Figs. 1–10.—*Achlya aplanes.*
Figs. 1, 2, 7, 8, 11.—Chlamydospores of various forms. (7) × 88; others
    × 145.
Figs. 3, 4.—Habit of zoosporangia. (3) × 88; (4) × 145.
Figs. 5, 6.—An oogonia with zoosporangia. × 145.
Fig. 9.—Habit of oogonia. × 145.
Fig. 10. An oogonium with diclinous antheridia. × 372.
Figs. 12–14.—*Achlya sp.* No. 1.
Figs. 12–14.—Various types of zoosporangia. × 140.
Figs. 15–16.—*Achlya sp.* No. 2.
Fig. 15.—Zoosporangia. × 83.
Fig. 16.—Zoosporangia. × 140.
Figs. 17–19.—*Achlya sp.* No. 3.
Figs. 17, 19.—Habit of zoosporangia. × 140.
Fig. 18.—Habit of chlamydospores. × 83.
Figs. 20–23.—*Achlya sp.* No. 4.
Figs. 20, 23.—Chlamydospores formed intercalary. × 83.
Figs. 21, 22.—Zoosporangia with apical papilla. × 140.
Figs. 24–29.—*Rhizidiomyces apophysatus.*
Figs. 24–25.—Sporangia of the parasite on oogonia of *A. Klebsiana.* × 610.
Fig. 26.—Spores resting on the oogonium. × 610.
Fig. 27.—Parasite sending rhizoids in the interior of the host.
Fig. 28.—Showing two empty zoosporangia of the parasite at (A).
Fig. 29.—A photomicrograph showing the parasite on the oogonia.

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P. L. Kochhar.

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H. Chaudhuri and
P. L. Kochhar.

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*II. Chaudhuri and
P. L. Kochhar.*

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P. *L. Kochhar.*

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P. L. Kochhar.

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P. L. Kochhar.

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ON THE STRUCTURE AND MECHANISM OF THE GASTRIC MILL IN DECAPODA.

VI. The Structure of the Gastric Mill in Natantous Macrura*—Penæidea and Stenopidea; Conclusion.

By S. S. PATWARDHAN, M.Sc.,

Department of Zoology, College of Science, Nagpur.

1. Introduction.

IN this paper I propose to describe the structure of the gastric mill in the only remaining tribes of Decapoda, *viz.*, Penæidea and Stenopidea. In the various forms of these tribes, the gastric mill presents appearances differing in degrees of complexity. In this concluding part of my work I propose also to give a historical account of the work so far done on the subject, a brief *modus operandi* of the gastric mill and a resumé of my entire work.

2. Structure of the Gastric Mill in Penæidea and Stenopidea.

(a) *Material and Method.*—The material at my disposal consists of the following species of Penæidea and Stenopidea obtained from the Biological Supplies stations at Ennur and Naples or from the Collections of the Zoological Survey of India, Calcutta.

Tribe : Penæidea.

Family : Penæidæ.

Penæus indicus M. Edw.[?]
Penæus semisulcatus Pettaan.[2]
Metapenæus affinis M. Edw.[1]
Penæopsis monoceros Fabr.[2]
Parapenæopsis stylifera M. Edw.[1]
Aristæus semidentatus sp. Bate.[2]
Haliporus æqualis sp. Bate.[2]

Family : Sergestidæ.

Acetes indicus M. Edw.[2]
Sergestes bisulcatus Wood Mason.[2]

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* I am aware that the arrangement of groups of Decapoda followed in these papers is obsolete. It has been followed merely for the convenience of description.

[1] From Ennur.

[2] From the collections of Zoological Survey of India.

Tribe: Stenopidea.

Family :: Stenopidæ.

Stenopus spinosus Risso.[3]

The foreguts were dissected and treated with a weak solution of caustic soda as I did in the other forms.

(*b*) *Foregut of* Penæus indicus *M. Edw.*—Reddy (1935) has given an excellent and well-illustrated description of the foregut of *P. indicus* and I shall, therefore, describe it very briefly.

From the roof of the œsophagus (Fig. 1, *t.*) project a group of six sharp spines, into its cavity. These spines, 'the œsophageal teeth' of Reddy, are interposed between the opposing surfaces of the molar processes of the mandibles. The cardiac stomach is elongated. Its floor is narrow and is separated from a pair of lateral folds (*m.f.*), one fold on each side, by a pair of deep ventral grooves. Each of the lateral folds bears a comb-like row of stiff setæ projecting over the ventral groove of its side.

The lower half of the lateral wall of the cardiac stomach is thick and calcified, forming an infero-lateral cardiac plate (*i.l.c.*) which bears a single longitudinal row of prominent denticles called "the supra lateral teeth" by Reddy. This plate is separated from the lateral fold of its side by a deep groove which is covered over by setæ borne on the lower edge of the infero-lateral cardiac plate. The mesocardiac ossicle (*m.c.*) is a small triangular curved plate with its apex pointing forwards (Reddy describes it as pointing backwards). Each of the paired pterocardiac ossicles (*pt.c.*) is narrow and elongated and runs obliquely downwards from the base of the mesocardiac ossicle. The pyloric ossicle (*p.*) is less calcified and membranous. It is a triangular piece with its apex pointing backwards. Each of the paired zygocardiac ossicles (*z.c.*) is roughly four-sided and projects into the cavity of the cardiac stomach in the form of a curved ridge. Anteriorly it is connected with the lower end of the pterocardiac ossicle and the posterior edge of the infero-lateral cardiac plate. Posteriorly it is attached to the pyloric ossicle. The median projection of the zygocardiac ossicle, into the cavity of the cardiac stomach, bears the lateral tooth consisting of a strong pointed anterior denticle followed by a single curved row of small denticles, deeply pigmented yellow or brown. The urocardiac ossicle (*u.c.*) is plate-like with its sides curved inwards. Its anterior end is conical and articulates with the mesocardiac ossicle and the pterocardiac ossicles, while its posterior end bears a median tooth (*m.t.*) consisting of a stout median

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[3] From Naples.
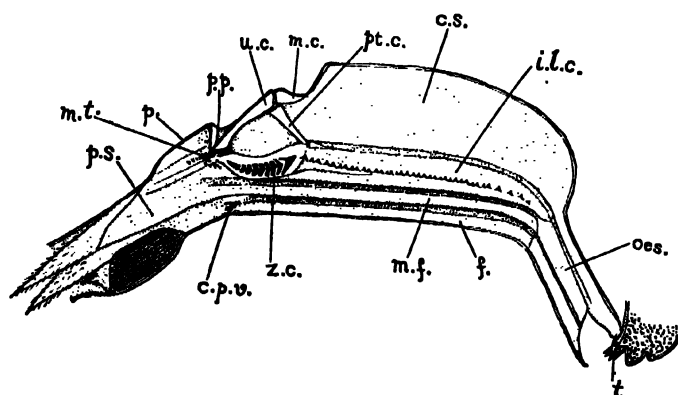
Fig. 1.

Fig. 2.
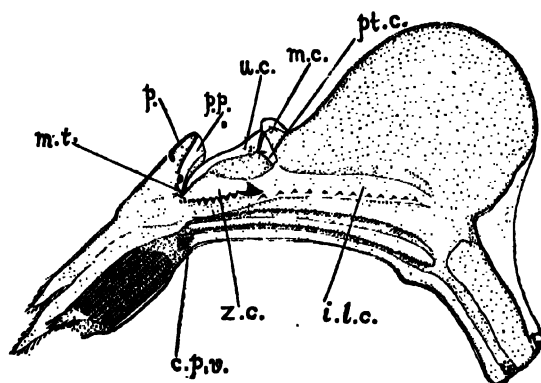
Fig. 3.

denticle at the apex and a row of small sharp denticles along the sides. The propyloric ossicle (*p.p.*) is triangular and curved (absent according to Reddy), with its apex pointing backwards and downwards and articulating with the lower end of the urocardiac ossicle. Its base is connected with the pyloric ossicle. The cardio-pyloric valve is wedge-shaped and membranous, covered

over with elongated setæ. The pyloric stomach is narrow but relatively longer than in the groups previously described (Patwardhan, 1935) in which the gastric mill is present. It is divided into two chambers, a dorsal and a ventral by a pair of lateral folds of its wall. The ventral chamber contains the characteristic filtering apparatus. The opening of the pyloric stomach into the midgut is guarded by two pairs of elongated valves, one pair on each side, and a small median ventral valve, all of which project backwards into the lumen of the midgut.

(c) *A comparative account of the gastric mill in Penæidea.*— The œsophageal teeth are present in all Penæidæ examined by me except in *Aristæus* and in Sergestidæ. The opening between the œsophagus and the cardiac stomach is not guarded by any special valves in Penæidæ but in Sergestidæ (Figs. 5 and 6) a pair of lateral triangular valves is present. The cardiac stomach is usually elongated as in *Penæus* but in *Aristæus*, *Haliporus* and *Sergestes* (Figs. 3, 4 and 6) the roof of the cardiac stomach is dilated anteriorly into a dome-shaped prominence. The floor of the cardiac stomach is generally similar to that found in *Penæus* except that in *Haliporus* (Fig. 4) the lateral fold is reduced to a ridge. The infero-lateral cardiac plate is apparently continuous with the zygocardiac ossicle of its side in those forms in which it bears a longitudinal row of denticles, *e.g.*, *Penæus*, *Aristæus* (Figs. 1 and 3), *Penæopsis*, *Metapenæus* and *Parapenæopsis*, while in *Haliporus*, *Acetes* and *Sergestes* (Figs. 4, 5 and 6) it is distinct from the zygocardiac ossicle. In *Acetes* and *Sergestes* the infero-lateral cardiac plate is covered over with elongated setæ. There is an additional lateral fold of the wall of the cardiac stomach situated dorsally to this plate in Sergestidæ.

The thickness and degree of calcification of ossicles, especially of those ossicles which bear the teeth and the prominence of the denticles forming a tooth are a measure, to a certain extent, of the efficiency of the gastric mill as an organ of mastication. Of the two families, Penæidæ and Sergestidæ, the latter possesses a more efficient gastric mill.

Among the Penæidæ, a number of deviations from a typical form are met with in *Aristæus* and *Haliporus* : the principal ossicles of the gastric mill are well differentiated but are thin and far less calcified than in the rest of the Penæidæ examined by me. In *Aristæus* the zygocardiac ossicle does not project to a great extent in the cavity of the cardiac stomach. The lateral tooth consists of a large anterior denticle (Fig. 3) followed by a single curved row of small denticles. The median tooth, however, is similar to one found in *Penæus*. In *Haliporus* (Fig. 4) the zygocardiac ossicle sends a pyramidal projection which bears anteriorly three vertically disposed large denticles and a curved row of small denticles as in *Aristæus*. The median
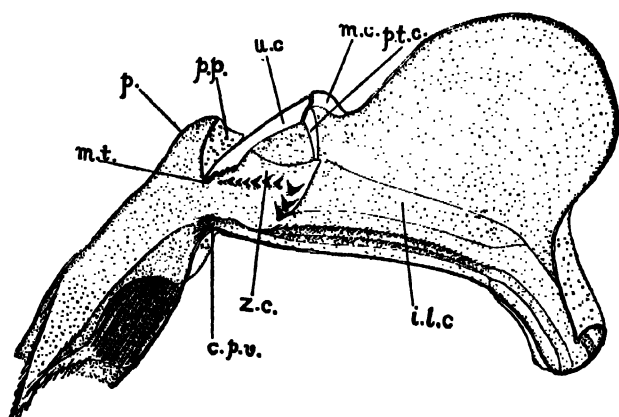
Fig. 4.
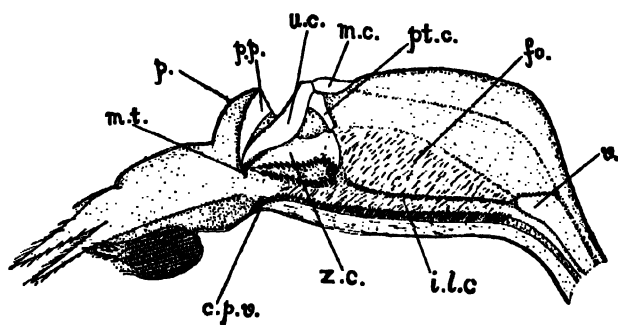
Fig. 5.
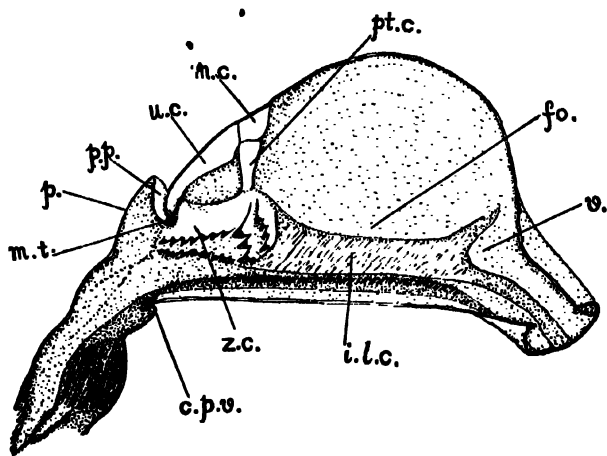
Fig. 6.

tooth is as in *Penæus*. In all the remaining Penæidæ examined by me, the structure of the gastric mill is more or less similar to the typical one of *Penæus* excepting the disposition of the' denticles on the lateral tooth, *e.g.*, *Penæopsis* (Fig. 2) in which the anterior large denticle is followed by a double curved row of small denticles.

In Sergestidæ, the ossicles of the gastric mill are relatively well differentiated, thick and densely calcified. The zygocardiac ossicle (Figs. 5 and 6) is very prominent and bears a thick broad projection into the cavity of the cardiac stomach. The lateral tooth consists of, in *Acetes* (Fig. 5) of three large anterior denticles which form a vertical row and a pair of curved rows of small denticles and in *Sergestes* (Fig. 6) of six anterior large denticles in two vertical rows, separated by a deep notch and a pair of curved rows of small denticles. The median tooth is similar to one found in *Penæus*, but relatively stouter. The large and more efficient lateral and median teeth may explain, to some extent, the absence of the rows of denticles on the infero-lateral cardiac plates in these two types.

The remaining ossicles in the forms examined by me are similar in shape but vary in size and in thickness and calcification in correlation with the nature of the zygocardiac ossicle.

(*d*) *The structure of the gastric mill in* Stenopus spinosus *Risso.—* An examination of the stomach of the *Stenopus spinosus* Risso. reveals that the œsophageal teeth are absent (Fig. 7) and the opening between the œsophagus and cardiac stomach is not guarded by any special valves. The cardiac stomach is not elongated as in Penæidæ or Sergestidæ but it is roughly box-shaped. The floor and the sides of the cardiac stomach resemble in structure those of *Penæus* except that the lateral folds are reduced to mere ridges and the infero-lateral cardiac plates do not bear longitudinal rows of denticles but each bears two short and curved rows of small denticles one behind the other, on its dorsal narrow portion. The various ossicles of the gastric mill have a definite outline but are not sufficiently calcified. The zygocardiac ossicle is not clearly distinguished from the rest of the wall of the cardiac stomach, the infero-lateral cardiac plate being continuous with it dorsally. The zygocardiac ossicle does not project into the cavity of the cardiac stomach to any appreciable extent. The lateral tooth consists of three anterior large denticles and five to six small posterior denticles. The urocardiac ossicle is broad and heart-shaped in outline and bears at its apex a curved median tooth. The broad urocardiac ossicle and the curved median tooth are of the same simple nature and have the same disposition as in *Cerataspis* (see Fig. 166, Calman, 1909) and in some Caridea (Patwardhan, 1935).
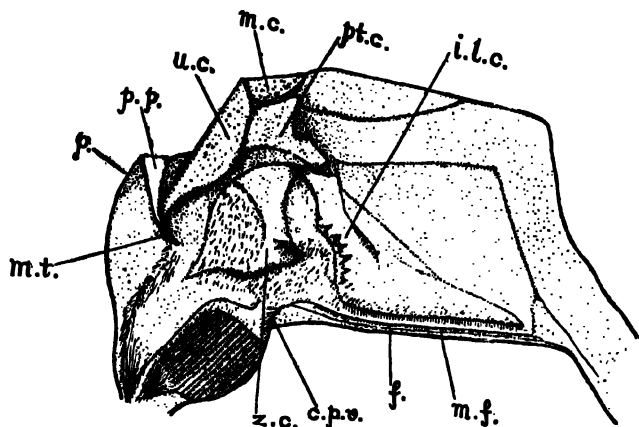
Fig. 7.

(e) *Structure of the mandibles.*—The mandibles in Penæidea and Stenopidea are intermediate in structure between the simple ones in Reptantia and the complex ones in Caridea. Typically the mandible (Fig. 8A) consists of a proximal hollow portion called the apophysis and a distal solid portion called the head comprising a sharp incisor process bearing on its superior or inner surface a molar process. The molar process is so reduced as to consist of a rounded prominence capped with a flat denticle deeply pigmented yellow or brown. The most interesting difference between the typical Caridian type of mandible as of *Palæmon* (Patwardhan, 1935) and of *Penæid* type, is the reduction in the latter group of the height of the molar process. In the Penæid group it does not extend beyond the cutting edge of the incisor process so as to make a deep cleft between the two processes. This levelling down of the molar process is a measure of its inefficiency for mastication, a function partly discharged by the gastric mill in this group.

In *Penæus, Penæopsis* (Fig. 8 *a* and *b*) and *Parapenæopsis* the cutting edge of the incisor process is serrated and in *Aristæus* and *Acetes* (Fig. 8 *c* and *e*) it is deeply cleft. The molar process bears a single flat denticle in *Penæus, Penæopsis* (Fig. 8 *a* and *b*), *Metapenæus* and *Parapenæopsis*, while in *Aristæus* it bears two denticles of unequal size (Fig. 8 *c*). In *Acetes, Sergestes* and *Lucifer* (Fig. 8 *e, f* and *g*) the masticatory surface of the molar process is fringed with a row of short spines.

In *Stenopus* (Fig. 8 *h*) the incisor process is deeply indented while the molar process is slightly more elevated and bears two sharply edged denticles of unequal size.

(f) *Discussion.*—Species of Penæidea and Stenopidea examined by me indicate that there is a gradation in the complexity of the structure of the
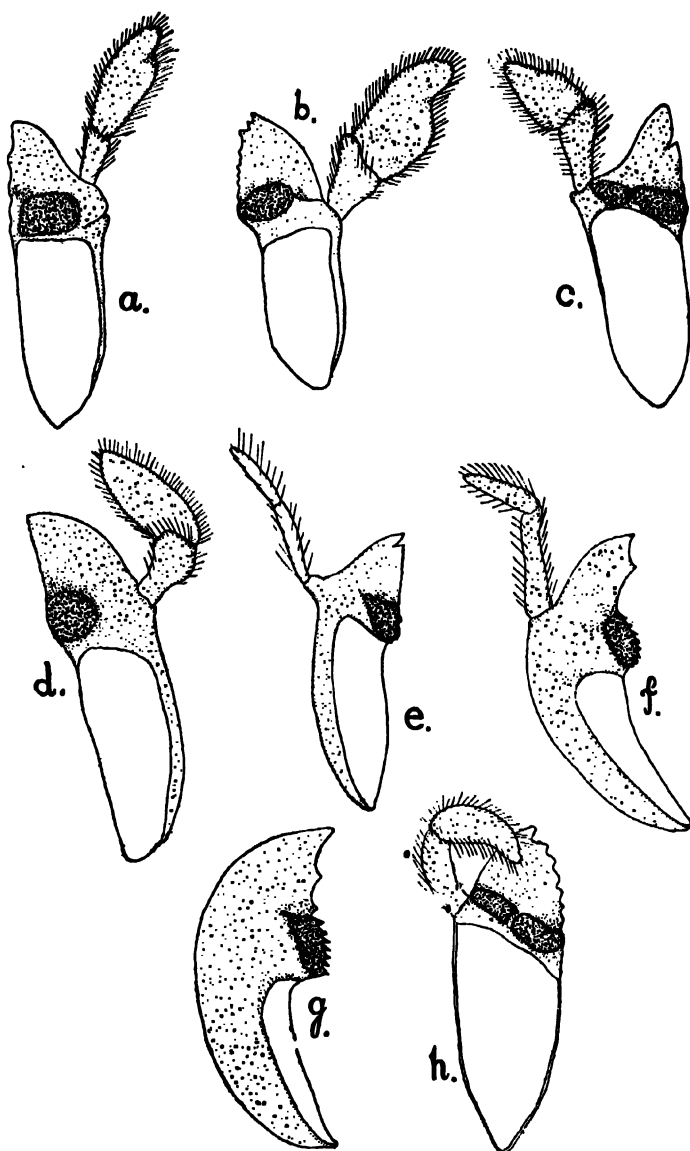
FIG. 8.

gastric mill, *Stenopus* having the simplest structure and the remaining forms
may be arranged in an ascending order of complexity as follows : *Aristæus*,
Haliporus, *Penæus*, *Penæopsis*, *Acetes* and *Sergestes. Cerataspis*, described
by Bonnier (1899) has the simplest form of gastric mill in the Penæid group

in which the ossicles of both the dorsal and the lateral series are absent (Calman, 1909). It is quite possible that the simple nature of the gastric mill in *Cerataspis* may be secondary and not primitive for the following reasons : we find that in *Stenopus* the gastric mill though simple is certainly more advanced than in *Cerataspis*. Taxonomically Stenopidea is more primitive than Penaeidea (Borradaile, 1907). A study of fossil remains of Crustacea indicate that representatives of Stenopidea appear earlier than those of Penaeidea or lower Reptantia (Calman, 1909 ; Zittel, 1927). From these considerations, it may not be wrong to regard the simple condition of gastric mill obtained in *Cerataspis* as derived by reduction of the gastric mill which was more complex. Such a reduction and disappearance of sclerites and consequent simplification of the gastric mill has been recorded in many Caridea (Calman, 1909 ; Patwardhan, 1935), in *Eupagurus* (Orton, 1927 and Nicol, 1932), in plankton feeding crabs, *Haplocarcinus* and *Cryptochirus* (Pott, 1915) and in *Lucifer* (Brook, 1882 and Rosenstadt, 1896), due to variations in the habit and habitat and the quality of the food of the animals.

It would be an interesting study to understand the reasons underlying the simplification and disappearance of the gastric mill in Caridea and Penaeidea. The reduction of the gastric mill is effected by two causes, the nature of the food material and the habit and habitat of the animals. The food material of the majority of Cardiea and Penaeidea is free from hard coverings of chitin or calcium salts and does not necessitate great labour in freeing the digestible from non-digestible portions of food. The external masticatory apparatus, chiefly the mandibles, are mainly concerned in grinding the food into a fine powder.

Apart from the quality of food, the swimming mode of locomotion of these animals gives them a certain amount of confidence which enables them to continue external mastication to a proper stage before the food is swallowed. A majority of Caridea, living in various depths, are good swimmers. Penaeid groups are particularly fond of shallow warm seas and in India they swarm the muddy waters of numerous deltas of the Bay of Bengal (Alcock, 1901).

3. *The modus operandi of the Gastric Mill.*

The urocardiac ossicle usually lies along the median line, slightly pressed downwards at its posterior end (Fig. 9a). The propyloric ossicle usually lies in an oblique position with its broad base directed anteriorly and the narrow lower end directed posteriorly and articulating with the lower and posterior end of the urocardiac ossicle.

Huxley (1880) regarded that both the anterior and posterior gastric muscles brought about movements of the median tooth. According to him,
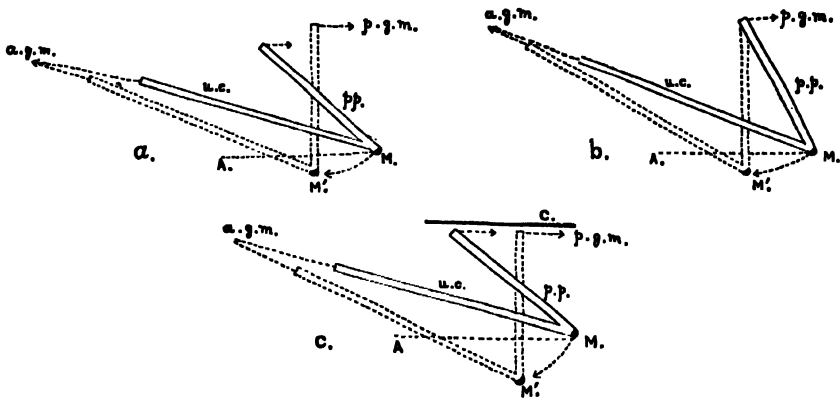
FIG. 9.
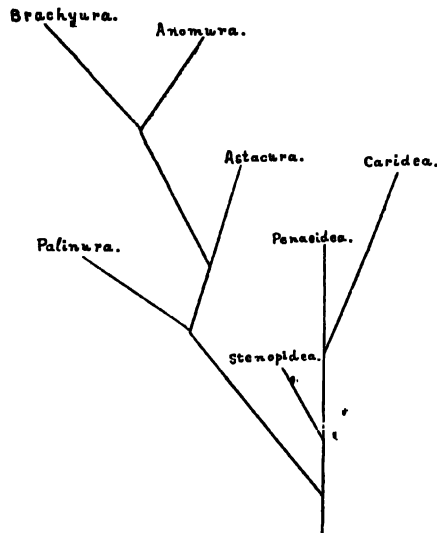
FIG. 10.

the anterior gastric muscles pull the urocardiac ossicle forwards which carries along with it the median tooth. Due to the oblique position of the propyloric ossicle the latter assumes a vertical position and in reaching this position it presses the median tooth downwards (Fig. 9a). So the movement of the median tooth instead of being in a straight line along AM, is along a curved path MM'. The posterior gastric muscles which pull the base of the propyloric ossicle backwards also help in making this ossicle vertical and thus share in pressing the median tooth downwards.

Mocquard (1883) confirmed the general disposition of the urocardiac and the propyloric ossicles as described by Huxley. Mocquard made his observations on a living crab *Stenorhynchus* in which the carapace was so transparent as to permit him to observe the action of the muscles. He states that the posterior gastric muscles have very little share in bringing about the vertical movement of the propyloric ossicle. They help only in keeping the ossicle in position while the contraction of the anterior gastric muscles alone pulls the urocardiac and also the lower end of the propyloric forwards (Fig. 9*b*). The oblique position of the propyloric ossicle is responsible for pressing the median tooth downwards while the ossicle assumes a vertical position during its forward movement.

Reddy (1935) on the other hand, thinks that the pressing down of the median tooth is due to the contraction of only the posterior gastric muscles which pull backwards the broad dorsal portion of the propyloric ossicle. This ossicle when it tries to be vertical, due to the presence of the roof of the cardiac chamber (?) (carapace) is pressed downwards (Fig. 9*c*) at its lower end.

Mocquard's view is generally accepted because he made his observations when the gastric armature was in action in a living crab, while all the alternative views are based on imagining the mechanical principles involved.

In *Gelasimus* (Reddy, 1935) the urocardiac and the propyloric ossicles show a unique disposition being in a straight line. In this position, neither the anterior nor the posterior gastric muscles can help to make the propyloric ossicle vertical and thus enable it to press down the median tooth. Reddy, therefore, is of opinion that in this animal, the cardio-pyloric constrictor muscles, unlike in other Crustacea, bring about the desired movements of the propyloric ossicles and consequently of the median tooth. He further states that in *Penaeus indicus* the movements of the median tooth are simply to and fro due to the absence of the propyloric ossicle. This is incorrect. The propyloric ossicle is present and lies in the same position as in other Decapoda, and helps the downward movements of the median tooth. Moreover, from the curved rows of denticles on the lateral tooth (Fig. 1) one may conclude that the movements of the median tooth must also be along a curved path, forwards and downwards, as in other forms.

4. Historical.

Probably the earliest mention of the gastric armature is made by Aristotle who mentions the teeth in the stomach of a crab, a fact from which it is generally inferred that he even practised dissections on lower animals. Geoffroy (1709) who repeated the observations of von Helmont and later on

Reaumur (1712) stated that every moult of *Astacus* always contained three teeth from the stomach. Rœsel (1755) was the first to remark that these teeth were useful for grinding food. The German naturalist Herbst (1790-1804) described the stomach teeth of *Homarus* and was the first to compare the median tooth with a bust mounted on a pedestal. Cuvier (1805) was the pioneer who in his *Leçons d'anatomie comparée* described clearly the principal sclerites of the gastric armature in Decapoda and also referred to their functions. About the same time as Cuvier, Meckel (1836) studied the gastric armature in *Palæmon, Squilla, Palinurus, Scyllarus* and a few other stalked-eyed Crustacea without giving any ossicle any definite designation. He was however the first to find gradations in the types of gastric armatures. Contemporary with Meckel were Brandt, Ratzeburg (1829) and Baer (1834) who worked on the gastric armature of Crustacea and gave a terminology which, though now obsolete, still marks a definite stage in the study of the gastric arinature. About the same time as Baer, who worked on Macrura, Milne Edwards in his *Histoire naturelle des Crustaces* (1834) gave a methodical and complete account of the gastric armature in the common crab and designated the various ossicles with simple names which were adopted by later authors, *e.g.*, Huxley, Parker and are used to this day. He came to an important conclusion, from the animals examined by him, that the disposition of the ossicles is essentially the same in all Decapoda. In his *Leçons sur la Physiologie et l'Anatomie comparée* (1834) this eminent naturalist compared the gastric armature with three branches meeting at a point through which food must pass to enter the pyloric stomach. He also referred to the specific and generic significance of the gastric armature. Oesterlen (1840) described the accessory ossicles of the gastric armature in great detail and with accuracy but his work is unfortunately of little value as he misunderstood the mechanical conditions of the gastric armature. It may be mentioned here that Joly (1843) and Leuckert (1848) described the stomachs of *Caridina* and *Mysis* respectively but ignored the fact that there is a typical gastric armature existing in different stages of variations in all Decapoda.

Huxley in his *Manual of the Anatomy of Invertebrate Animals* (1877) and later in *Crayfish* (1880) examined the movements of the stomach teeth and likened them to a "mill" and the pyloric stomach to a "filter". Parker (1876) working on the same animal described the muscles of the stomach which bring about movements of the different ossicles.

In 1880, Nauck published his *Memoir on the Gastric Armature of Brachyura*. He proposed a new classification of the group and a new nomenclature of the ossicles but these did not find favour with the later authors. In 1883 and 1884, appeared a monumental paper by Mocquard on the stomach of

the stalked-eyed Crustacea. He gave an excellent historical note of the work so far done on the subject. He examined about a hundred species and came to the conclusion that the gastric armature showed a uniform plan of construction and varied only in the coalescence and disappearance of one or more of the ossicles, while no new piece was ever added to the general framework and even in the most degraded forms the homologies of the pieces could be recognised. From this study of gastric armature he was first to find the artificial nature of the classification of Decapoda which was later modified, on other grounds, by Borradaile (1907). He further described the musculature and its actions which he was able to observe in a living crab, *Stenorhynchus*.

Mocquard's classical paper was followed by a number of detailed studies of gastric armature in different species of Crustacea, *e.g.*, Bonnier (1899) on *Cerataspis*, a member of Penæid group which possesses the simplest form of gastric armature, Claus (1899) on *Nebalia*, Meek (1903) on the Norway Lobster, Borradaile (1904) on a "robber crab", Pearson (1908) on *Cancer*, Jackson (1913) on *Eupagurus*, Petricevie (1915) on *Squilla*, Matteotti (1921) on *Potaman*, Yonge (1924) again on Norway Lobster and Reddy (1935) on some South Indian Decapoda and *Squilla* with a discussion of its evolution in Decapoda.

In addition to these, general treatises on the class Crustacea, *e.g.*, Calman's indispensable volume in Lankester's *Treatise on Zoology* (1909) giving a concise summary of the work so far done on the stomach teeth of Crustacea, Winterstein (1911) and Kukenthal (1926-27) give an excellent account of the gastric armature and its working in Crustacea.

5. *Conclusions.*

The present work was undertaken with a view :—

(1) to study the structure of the gastric mill in the Decapoda and the disposition and the homologies of the ossicles forming the gastric armature ;

(2) to arrange the various types of the gastric mills found amongst the Decapoda in a series beginning from very simple to complex ;

(3) to ascertain if the forms considered as "primitive" amongst the Decapoda for taxonomic and other reasons possess a simpler type of gastric mill as compared to the gastric mill of the more "advanced" forms ; and

(4) to correlate the efficiency of the internal masticatory apparatus with that of the external masticatory apparatus, chiefly the mandibles.

(1) I commenced this work with a detailed description of the foregut of *Paratelphusa guerini* M. Edw. which contains a highly complex gastric mill. In the next paper, I gave a comparative account of the gastric mill in various forms of Brachyura. Unfortunately, I was not fully conversant with the work of Mocquard (1883) till I sent that paper to the press. It contains nevertheless an account of the gastric mill of many new forms not described by Mocquard. Reddy (1935) gave an account of the gastric mill of *Paratelphusa hydrodromus*, Herbst. In this and his other paper (1935), there has been unfortunately a certain amount of work on the types also worked by me. But as both the papers were in the press at the same time this was unavoidable.

An examination of fifty species of Decapoda confirms Mocquard's view that the gastric armature in Decapoda, when it is present, conforms to a typical plan which is common to all species. The variations in the gastric armature in the various types are due to the differences, which are of generic and specific significance, in shape and size of the ossicles, coalescence, reduction or disappearance of one or more of them. For instance, the mesocardiac ossicle is relatively smaller in those forms in which the gastric mill is very complex, *e.g.*, in all Brachyura and in *Paguristes* amongst Anomura while in a few Anomura, Reptantous Macrura and Penæidea it is comparatively smaller (Patwardhan, 1935). The pterocardiac ossicles vary in shape and size inversely to that of the mesocardiac ossicle. The pyloric ossicle is strongly calcified in the complex gastric mills of Brachyura and may present a paired appearance due to deficiency of calcification in the middle. In the remaining types it is more or less uniform in structure, getting less calcified in simpler forms as in Penæidea. The exopyloric ossicles are present in Brachyura and a few Anomura (Patwardhan, 1935), but are coalesced and fused with the pyloric in the remaining groups. The zygocardiac ossicles vary in thickness and present varieties of disposition of the denticles forming the lateral teeth, being larger and stronger in the complex forms and reduced to spines in the simpler forms. The urocardiac ossicle is always elongated, narrow and rod-like in complex forms, *e.g.*, Brachyura and Anomura and gets plate-like and broader in the simpler ones. The median tooth in its most complex form consists of several large denticles variously grouped ; in Anomura it bears transverse ridges while in the simpler forms it usually consists of a large median tooth with or without small lateral denticles. The propyloric ossicle which is always more or less triangular in shape, and oblique in disposition, is usually well calcified but may remain membranous in the middle. The cardiac pyloric valve, sometimes referred to as a median ventral tooth, is wedge-shaped and covered with setæ in many Brachyura, Reptantous Macrura and Penæidea

but in Anomura and a few Brachyura it is distinctly calcified and bears tooth-like denticles.

In those Decapoda in which the gastric mill is absent, *e.g.*, the majority of Caridea, it is possible to homologise the structures around the cardiac pyloric opening with the principal ossicles of a typical gastric mill, while in Atyidæ and Hippolytidæ only the median tooth is present together with the median set of ossicles (Calman, 1909).

(2) If the presence of a complete set of ossicles in a gastric mill, their relative thickness and calcification and the degree of stoutness of the teeth are a measure of the efficiency of a gastric mill, it is possible to arrange the various types found amongst the Decapoda in a graded series of complexity. In Caridea, excepting Hippolytidæ and Atyidæ, the gastric mill is entirely absent. It is present in all other groups of Decapoda. The simplest gastric mill is found in *Cerataspis* (Bonnier, 1899 ; Calman, 1909) and a slightly more complex one in *Stenopus*. The structure becomes increasingly complex in *Aristæus*, *Penæus* and *Sergestes*. Next in the series come the Reptantous Macrura, *e.g.*, Palinuridæ, Scyllaridæ and Astacura. The most complex gastric mills are found in Anomura and Brachyura, especially in the tribes Cyclometopa and Catametopa of Brachyura.

(3) According to Borradaile (1907), the affinities of the various groups of Decapoda are represented by Fig. 10. Stenopidea represents the lowest form of Decapoda. On one side we get the Penæidea and Caridea comprising Natantia and on the other, the lower Reptantia which subsequently branch into Astacura, Anomura and Brachyura.

The above relationship is confirmed by a study of the structure of the gastric mill in the various groups. The Stenopidea is characterised by the possession of a very simple gastric mill. It is true that the gastric mill in *Cerataspis* in the Penæidea is still simpler and therefore *Cerataspis* may be treated as being exceptional amongst the Penæidea. In all probability, the simplification in *Cerataspis* may be secondary. From the Stenopidea the evolution proceeds along two directions :

(*a*) complexity in the gastric mill resulting in the condition to be found in Penæidea ; from this complexity the further evolution along this direction is towards the reduction of the gastric mill in Hippolytidæ and Atyidæ resulting in its total absence in the remaining Caridea ;

(*b*) starting again from the Stenopidea, the evolution proceeds towards a progressive increase in the complexity of the gastric mill resulting in a graded condition found in Lower Reptantia, Astacura, Anomura and Brachyura. As we ascend this series, the ossicles get more distinct, the

exopyloric ossicles separate from the pyloric, the mesocardiac gets smaller, the pterocardiac ossicles become more elongated and the lateral and median teeth become more complex.

(1) I have described briefly the structure of the mandibles in all the groups of Decapoda. It is possible to correlate the efficiency of the internal masticatory apparatus, *viz.*, the gastric mill with the external masticatory apparatus chiefly the mandibles. In Brachyura, Anomura and Reptantous Macrura, where the internal mastication is efficient due to the presence of gastric mill, the mandibles in these groups show a much simpler structure than what is obtained in the Stenopidea, Penæidea and Caridea. In the latter groups the internal masticatory apparatus is either totally absent or so modified as to be in the form of a few denticles on the various parts of the foregut. The molar processes of the mandibles are chiefly concerned with grinding the food into a fine powder. The stoutness, length and the disposition of the denticles on this process are a measure of the degree of the efficiency of the mandible as a masticatory apparatus. The majority of Caridea, in which the internal mastication is minimum, have the most complex mandibles (Patwardhan, 1935). In the Reptantia, in which the gastric mill is very complex, the molar process is greatly reduced, being always in the form of a small prominence without any masticatory function. The condition of the molar processes in the Stenopidea, Penæidea and Atyidæ and Hippolytidæ in Caridea, which have simple gastric mills, is intermediate in character between the Caridea and Reptantia, being flattened and less prominent than in Caridea but still serviceable as masticatory organs.

Combining the results of the last two paragraphs, it may be said that Reptantia as a group is characterised by the presence of a more or less complex gastric mill and simple mandibles and Natantia, on the other hand, is characterised by the presence of a more or less simple gastric mill and complex mandibles always possessing a functional molar process.

The reason for the presence of internal masticatory apparatus in one case and of external masticatory apparatus in another must be sought in the habit and habitat of the animal concerned. From a study of the habits and modes of locomotion in the Decapoda, it will be seen that the " Natantous habit" has a wider field and ampler prospect of obtaining food, more amount of confidence and boldness which enable these animals to continue external mastication to its proper stage without necessitating further grinding in the cardiac stomach after it is swallowed. On the other hand, "Reptantous habit" which means slow locomotion by crawling and climbing, a smaller field for obtaining food and a wary and nervous life, necessitating masking and hiding adaptations results in a hurried swallowing of food without its

proper mastication by the external masticatory apparatus, thus necessitating internal mastication.

The quality of the food material must also influence the complexity of the masticatory apparatus. Food of the crabs and other Reptantia is usually encased in non-digestible chitinous or calcareous envelopes which must be removed before the food is cut into suitably sized morsels for swallowing. The external masticatory apparatus is greatly used in crushing the food and separating the digestible from the non-digestible, the proper mastication being deferred to be carried out by the gastric mill. The individual variations in the complexity of the gastric armature or of the molar processes of the mandibles are no doubt due to the quality of the food which the animal prefers. But it is not possible to come to any definite conclusions unless and until a detailed study of the food in each animal is made.

My thanks are due to the Director, Zoological Survey of India, for identifying the material at my disposal, for giving me material from the collections of the Zoological Survey and for the loan of books from the Library.

Summary.

A brief description of the foregut in *Penæus indicus* and a comparative account of the same in the remaining types of Penæidea and Stenopidea examined by me has been given.

The structure of the gastric mill in the Decapoda conforms to a typical plan common to all species, the variations being due to the coalescence, reduction or disappearance of one or more ossicles; in those forms in which the gastric mill is reduced the homologies of the principal ossicles can be made out.

The various types of the gastric mills found in the Decapoda can be arranged in a series ranging from simple to complex.

The forms considered as "primitive" for taxonomic and other reasons possess a simple type of gastric mill as compared to the gastric mills in the more "advanced" forms. The evolution of the gastric mill closely follows the evolution of the Decapoda.

The efficiency of the gastric mill is correlated with the efficiency of the external masticatory apparatus, chiefly the mandibles, the mandibles being simple in the forms in which the gastric mill is complex and *vice versa*.

The reason for the presence of the internal masticatory apparatus in one case and of the external masticatory apparatus in the other can be found in the habit and habitat of the animals. Reptantous habit is associated with the possession of a complex gastric mill and simple mandibles and the Natantous habit, on the other hand, with the possession of a reduced gastric mill and complex mandibles.

B3   F

EXPLANATION OF FIGURES.

Fig. 1. –Vertical longitudinal section of the foregut of *Penæus indicus* M. Edw., passing through the median plane.

Fig. 2. –*Penæopsis monoceros* Fabr. Disposition of the denticles of the lateral tooth.

Fig. 3. Vertical longitudinal section of the foregut of *Aristæus semidentatus* sp. Bate., passing through the median plane.

Fig. 4.—Vertical longitudinal section of the foregut of *Haliporus æqualis* sp. Bate., passing through the median plane.

Fig. 5.—Vertical longitudinal section of the foregut of *Acetes indicus* M. Edw., passing through the median plane.

Fig. 6.—Vertical longitudinal section of the foregut of *Sergestes bisulcatus* Wood Mason, passing through the median plane.

Fig. 7.—Vertical longitudinal section of the foregut of *Stenopus spinosus* Risso., passing through the median plane.

Fig. 8. The Mandibles of—

     A. *Penæus indicus.*
     B. *Penæopsis monoceros.*
     C. *Aristæus semidentatus.*
     D. *Haliporus æqualis.*
     E. *Acetes indicus.*
     F. *Sergestes bisulcatus.*
     G. *Lucifer hanseni* Nobili.
     H. *Stenopus spinosus.*

Fig. 9.—

     A. To illustrate the movement of the median tooth according to Huxley, 1880.
     B. To illustrate the movement of the median tooth according to Mocquard, 1883.
     C. To illustrate the movement of the median tooth according to Reddy, 1935.

Fig. 10. Evolution of Decapoda. After Borradaile, 1907.

REFERENCE LETTERS.

a.g.m.	..	Anterior gastric muscles.
c.	..	Carapace.
c.p.v.	..	Cardio-pyloric valve
f.	..	Floor of cardiac stomach.
fo.	..	Fold of the lateral wall of cardiac stomach.
i.l.c.	..	Infero-lateral cardiac plate.
m.c.	..	Mesocardiac ossicle.
m.f.	..	Lateral fold of the floor of cardiac stomach.
m.t.	..	Median tooth.
oes.	..	Oesophagus.
p.	..	Pyloric ossicle.
p.g.m.	..	Posterior gastric muscles.

p.p.	..	Propyloric ossicle.
p.s.	..	Pyloric stomach.
pt.c.	..	Pterocardiac ossicle.
t.	..	Oesophageal teeth.
u.c.	..	Urocardiac ossicle.
v.	..	Valve at the opening of oesophagus into the cardiac stomach.
z.c.	..	Zygocardiac ossicle.

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THE STRUCTURE AND LIFE-HISTORY OF *AZOLLA PINNATA* R. BROWN WITH REMARKS ON THE FOSSIL HISTORY OF THE HYDROPTERIDEÆ.

By H. S. RAO, M.Sc.,

Department of Botany, University of Lucknow.

THE genus *Azolla* was instituted by Lamarck in 1783 on sterile specimens brought from Magellan by M. Commerson (Griffith, 1849, p. 556). It comprises at least four living species, *A. filiculoides, A. caroliniana, A. pinnata* and *A. nilotica*; there is a fifth species, *A. rubra,* which Strasburger has kept only as a variety of *A. filiculoides.* There are three species of the genus, *A. tertiaria* Berry (1927, 4-5, Pl. I, Figs. 9, 10), *A. intertrappea* Sahni and Rao (1934, 26, 27), and *A. prisca* Reid and Chandler (1926), which are known only in the fossil state. Both *A. filiculoides* and *A. pinnata* are also known in the fossil state (Florschütz, 1928).

The genus has representatives in all the divisions of the globe at the present day. *A. filiculoides* is confined to the western part of America, being reported from as far south as Chile, and reaching to California at least, and probably beyond (Campbell, 1893, 155). It ascends in the Andes to 16,000 ft. (Baker, 1887, 137). *A. rubra* is found in Australia and New Zealand. *A. caroliniana* is found in the southern United States and California, through tropical America to Buenos Ayres. *A. pinnata* is the type in Australia; the variety, which approximates in habit to *A. caroliniana,* is widely spread in tropical Asia and Africa. *A. nilotica* is exclusively African. The accompanying map shows approximately the distribution of the living and fossil species.

Thus the only living species known in India is *A. pinnata.* This species, with *A. nilotica,* belongs to the section Rhizosperma; all the other living species are included in Euazolla. Of the fossil species *A. intertrappea* belongs to Euazolla, *A. prisca* combines the characters of Euazolla and Rhizosperma, and *A. tertiaria* is of doubtful affinity, being incompletely preserved.

Historical Review.

The genus *Azolla* has been described by a number of workers: Griffith,[*] Mettenius, Mayen, Martius, Strasburger,[*] Berggren[*], Belajeff, Campbell[*] and others. Of these, the works of Strasburger, Berggren and Campbell are by

H. S. Rao

LIVING AZOLLA
A. caroliniana.
A. filiculoides
A. rubra
A. nilotica
A. pinnata

FOSSIL AZOLLA
■ A. intertrappea
× A. prisca
○ A. tertiaria
● { A. filiculoides
      A. pinnata
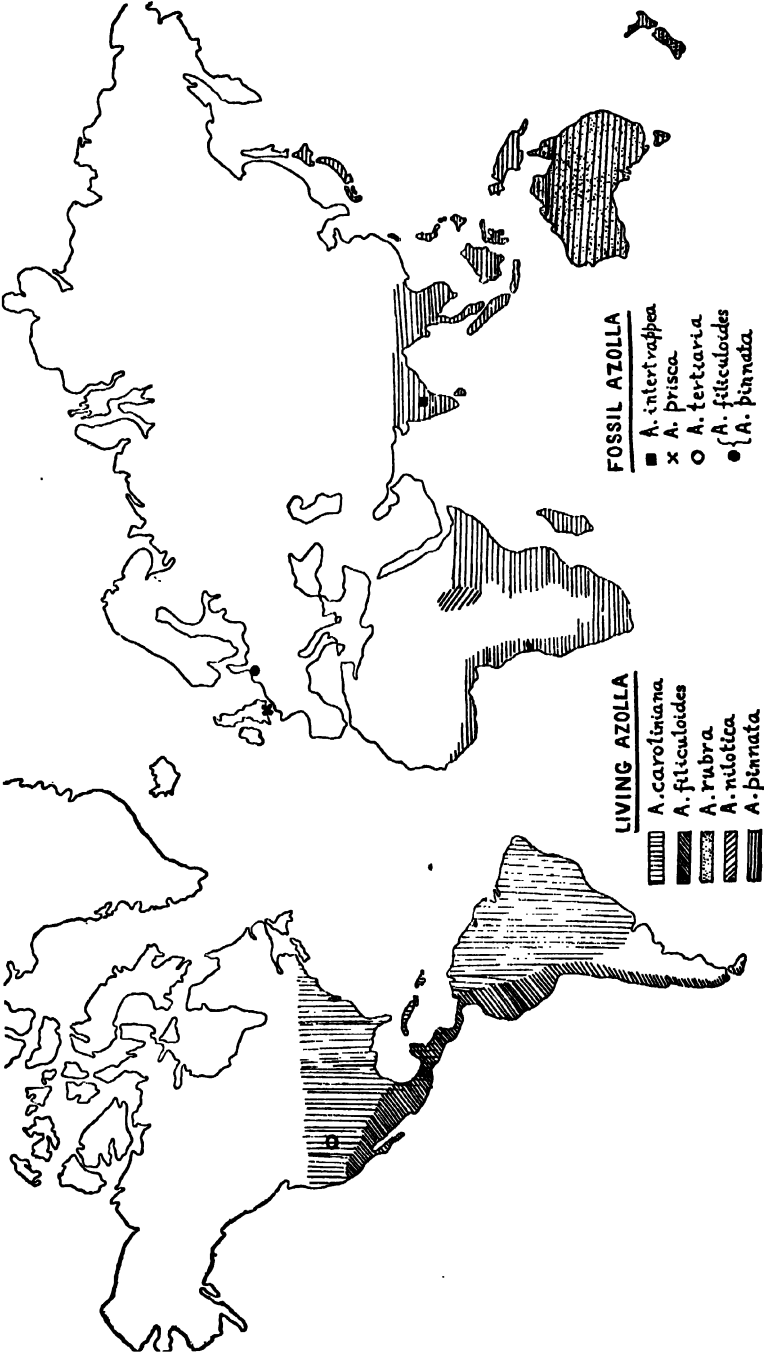
far the most important. The results of the earlier workers have been given by Strasburger in his monograph. Of the above-mentioned works only those marked with an asterisk have been available to me.

Griffith's work (Griffith, 1884) has only a historical interest. He has given a large number of drawings of the sporocarps of *A. pinnata*. Obviously the sporocarps were a great puzzle to him. His nomenclature for the various organs is archaic, often faulty. His was the earliest work on the Indian species.

Strasburger's monograph of the genus (Strasburger, 1873) although now sixty-four years old, still commands respect as an authentic record of observations, like most of his other works. The details of structure were elucidated chiefly in *A. filiculoides* though other species were also figured. His work deals exhaustively with the anatomy and histology of the mature sporophyte, and also of the full-grown sporangium and spores. He could not study the development of the spores for lack of material.

Berggren's paper (Berggren) deals with the female prothallium and embryo. His figures are mostly rather diagrammatic. Campbell's work (1893, 1928) largely supersedes this, being more complete. It is very important ; he has not only summarised the results of the previous workers, but also worked out in detail the life-history of *Azolla filiculoides*, which is thus the best known species.

Pandit and Mulay (1931, 267) who published a preliminary note on the *Azolla* found at Khandala in the Western Ghats (Bombay Presidency) suggest that their plant is a new species or a new sub-genus. This view is no doubt due to their ignorance of the literature. Their account shows that their plant is in no way different from *A. pinnata*.

Mr. Sud's preliminary note (1934, 189 196) on *A. pinnata* is also incorrect in several points, *viz.*, branching of stem, growth of leaf, the time of fertilisation, and the female prothallus (see below under Discussion).

I took up the investigation of the structure and life-history of the Indian species at the kind suggestion of Prof. B. Sahni. A necessity was felt for a comprehensive account of at least one species, other than *A. filiculoides*, if only to confirm the results arrived at for the latter. *A. pinnata* seemed specially suited for such a study, as it belongs to the second sub-genus Rhizosperma.

My observations, though not so complete as Campbell's on *A. filiculoides*, have, however, shown that *A. pinnata* resembles that species in almost all its salient characters.

The basis on which the two sub-genera Euazolla and Rhizosperma have been formulated is the characters of the float-corpuscles and the glochidia. The diagnosis of Rhizosperma and *A. pinnata* given by Baker is incorrect in one particular. He says the roots are fascicled in *A. pinnata*. Strasburger's diagnosis and drawing show that they are single in *A. pinnata*, which my observations also confirm, and fascicled only in *A. nilotica*.

An important point elucidated in my investigations is that the male prothallia of *A. pinnata* are liberated from the massulæ.

Material and Methods.

First of all a few specimens from Cuttack sent by Prof. Parija were examined ; next I examined material from Poona sent to Prof. Sahni by Mr. B. R. Pandit, some from Sagar, Mysore State, collected by me, and also some from Agra sent by Mr. B. M. Johri. Having ascertained that there is only one species known so far in India, further work was conducted on plants collected at Lucknow. The structure was studied mainly by microtoming the entire plants, the small size of the plants being convenient to handle that way. Dissection under the binocular and dissecting microscopes was illuminating. As fixatives I used chrom-acetic acid and sometimes formalin-acetic-alcohol. To sink the plants in the fixative I had to use the exhaust pump. Safranin and gentian violet gave as good staining results as iron-alum hæmatoxylin.

The megasporocarps gave the greatest trouble in cutting. The spore wall is very tough and hard like the chitin of insects. Softening in hydrofluoric acid was helpful to some extent but the innermost spore-wall was almost always broken.

Description.

Azolla is common in the United Provinces, occurring abundantly in tanks and pools. The "fronds" are triangular or polygonal in shape, measuring about 10 mm. to 25 mm. on the sides (Figs. 1–4). The stem is dorsi-ventral, bearing dorso-laterally two rows of alternate leaves, which are deeply two-lobed. The branching is in a scorpioid cymose manner. At each forking of the stem generally a root hangs downwards, looking feathered on account of the long root-hairs, whence the specific name. The sporocarps are formed from the ventral lobe of the first leaf of a branch. After a period of vegetative multiplication by fragmentations of the plants, a normal sexual regeneration occurs. The diagnosis of the Indian plant is as follows :—

Azolla pinnata R. Brown.—Roots single and conspicuously feathered on account of the root-hairs. Fronds oblong or deltoid, 10 mm. to 25 mm.

long, with numerous crowded primary branches, all simple or the longest with a few crowded primary branches towards the tip. Leaf lobes firm in texture, broad-ovate. Macrospore crowned with nine float-corpuscles, its cuticle finely granular, armed with a few clavate papillæ. Massulæ more than two in the microsporangium, with only a few weak simple or branched processes on one side (Baker, 1887, 138 ; Strasburger, 1873, 79).

A. nilotica differs from *A. pinnata*, along with which it is grouped in the sub-genus Rhizosperma, by its wide trailing leafless stem, with dense fascicles of roots from its nodes. The sub-genus Euazolla, under which are grouped *A. filiculoides* and its var. *rubra*, and *A. caroliniana*, differs from Rhizosperma in having the macrospores crowned with three float-corpuscles ; massulæ armed all round with rigid glochidiate processes.

As far as my observations show, the life-history of *A. pinnata* at Lucknow runs roughly as follows : Fresh embryo plants come up about September or October. They are green at first, but acquire a brownish colour in the cold season on account of a pigment formed in the leaves. The plants produced from the sporocarps mature in spring. By about April the sporocarps are ripe and are separated from the mother plants, which soon die off. During summer the plants are almost completely absent except some which remain vegetating where there is deeper water. A normal sexual method of regeneration occurs after the rains, *i.e.*, at about the end of September. During the vegetative season—September to April—the plants rapidly multiply by fragmentation. This happens both through the mechanical action of the waves, and by the insects, snails and other animals nibbling at the plants. This is further facilitated by the formation of an absciss layer (Fig. 20) at the bases of the branches. There is a similar absciss layer of smaller cells at the bases of the roots (Fig. 27) and hence they are deciduous when mature. •

Stem.

The stem apex is curved upward and backward (Fig. 11) reminding us of the circinate vernation of the fern leaf. There is a two-sided apical cell from which segments arise right and left. The first division of the apical cell is horizontal, *i.e.*, parallel to the upper and lower surfaces (Fig. 11), the second in the sagittal plane. A transverse section through the stem at this stage shows four cells like the quadrants of a circle. The dorsal segments develop the leaves, the ventral ones the lateral branches and roots. Strasburger's drawings show with great clearness the successive divisions at the stem apex. The third division forms the octant walls. The fourth one is periclinal and lays down the central cell-complex which develops the conducting strands.

The mature stem shows a central vascular bundle (Fig. 10). The structure is very simple, there being only three or four spiral tracheids and a few phloem elements. The endodermis is clearly seen, though the Casparian thickenings are not marked. There are only a few air-spaces in the cortex, which open out by means of stomata (Fig. 12). Aeration of the plants is more efficiently carried out by the leaves.

Branching is extra-axillary, *i.e.*, having no relation with the leaf axils. The lateral branches arise in acropetal order, but not always at equal intervals. Their development is a repetition of the main axis. At the bases of the branches there is an absciss layer composed of smaller cells. This does not seem to have been noticed by the previous workers. On account of this the plants easily fragment at these places (Fig. 20).

The stem, like the dorsal lobes of the leaves, is covered by hairs (Fig. 12). At the stem apex, among the lateral organs, are found a few large hairs (Fig. 11) with a large basal cell, bearing branches at the distal end. Tangles of *Anabæna* are always found at the stem apex and enter the leaf-cavities while these are being formed.

The dorsal lobes of the leaves so closely overlap each other like the tiles of a roof, that the stem is not visible when viewed from above. The under-surface is similarly covered by the lower lobes (Figs. 2, 4).

Leaf.

The leaf arises from the upper segments of the stem quadrant, as described by Strasburger and Campbell in *A. filiculoides*. Only the alternate cells on the right and left produce leaves, resulting in alternate rows of leaves. Thus, though the apical segmentation is regular, there is no constant relation between the formation of the segments and the origin of the appendages. This is the case in all Pteridophytes where the apical segmentation is most regular (Bower, 1908, 177). The mother-cell of a leaf is distinguished by its size and position. The first wall divides it into an inner small cell and an outer larger cell. The latter forms the upper and the former the lower lobe of the leaf. Each leaf-lobe is next divided into an inner and an outer cell. The latter divides into two by a radial wall. Then succeed alternate radial and tangential walls which are repeated with great regularity and can be seen in the young stages of the leaf (Fig. 7). No apical cell in the leaf is produced in *Azolla*, an exceptional feature in ferns.

An invagination is formed in the dorsal lobe at this stage. *Anabæna* cells already begin to enter it. This invagination (Fig. 14) later becomes the *Anabæna*-cavity of the leaf. The wall of the cavity bears a number of characteristic large hairs, which consist of a basal cell like the holdfast of

an alga, from the bulged distal end of which generally a few-celled branch arises at a rather small angle (Figs. 16-19).

The mature leaf is deeply two-lobed (Fig. 13). The lower lobe covers the stem on the ventral side, and is in contact with the water. It is simple in structure, one-layered for the most part except in the middle near the base, where it becomes a few cells thick and contains pigment. There is a small cavity in the thickness of this lobe near the base. This, however, does not open on the outside, and encloses no *Anabæna* (Figs. 21, 22). The ventral lobe is broader than the dorsal.

The dorsal lobe of the leaf is aerial. It has a complex structure (Figs. 13, 21, 22). It is ovate in shape, thick and firm. The dorsal lobes overlap each other like the tiles of a roof. The lobe is thick in the middle, with a thin colourless margin, one cell thick and 4 to 5 cells broad. The upper surface of the leaf is covered by turgid, colourless, two-to four-celled hairs, borne obliquely forward and upward. These hairs hold air-bubbles among them, and thus the plants cannot be made to sink at all. Even an exhaust pump does not make the plants sink readily.

Both the upper and lower surfaces of the dorsal lobe bear a number of stomata, whose form is shown in Figs. 13 and 15. These are peculiar, simple perforate cells as described by Sud, the pore being slitlike, reminding one of the stomata on the apophysis of *Funaria* (Campbell, 1928, 110, Fig. 239 *b*).

There is a peculiar, large, hollow cavity in the dorsal lobe of the leaf, which opens by an aperture on the lower surface (Figs. 13, 14, 21, 22). As mentioned above, it is formed as an invagination in the leaf-lobe into which *Anabæna* cells creep in. When the leaf matures the cavity becomes larger, its mouth narrows down to a small aperture, somewhat elongated in the longitudinal direction. The aperture is lined by three rings of tooth-shaped cells (Fig. 21). Thus it reminds one somewhat of the stomatal apertures of *Marchantia*. The wall of the cavity around the aperture is formed of two layers of elongated, colourless cells, radiating from the aperture (Fig. 13). The layer of cells lining the cavity bears a few hairs (Figs. 16-19), which consist of a basal cell like an algal holdfast, the bulged distal end of which bears one or more branches, a few cells in length. These branches hold the *Anabæna* tangles, which do not immediately line the cavity, but are held a little way towards the centre by these hairs.

The upper epidermis of the dorsal lobe, as mentioned above, bears stomata. These open into air-spaces interspersed in the palisade tissue. The latter is usually one layer thick. Its cells contain chloroplasts lining its walls. Below the palisade is the mesophyll tissue, which also lines the

Anabæna cavity. There is only a feeble development of conducting elements in the leaf, represented by only a few spirally thickened tracheids.

The leaves are green early in the season. By about November the plants turn brownish red on account of the red pigment produced in the cells of the leaf.

Root.

The root is produced from the lower segments of the stem, resulting from the quadrant division. The root initial cuts off a cell superficially which again divides into two. There is an oblique wall formed in the initial, cutting off the apical cell. After the next three divisions, the three-sided pyramidal apical cell is already formed, with the outer wall bulging out. When only two of these divisions have taken place, a transverse section of the apical cell is approximately 4-sided.

Azolla is distinct from other Filices in the mode of development of the root-cap (Strasburger, 1873, 44–46, and Figs. 53–72, Pls. 4 and 5). The first superficial cell cut off by the root initial forms two root-sheaths, the inner of which disorganises. Before the disorganisation, however, the apical cell cuts off two layers of cells, so that at this stage the apical cell is enveloped by four layers which are later reduced to three by the disorganisation of the inner root-sheath (Figs. 23, 29, 31). Strasburger too showed this feature clearly.

In the nearly adult root two root-sheaths are still found covering the root-tip, but they were broken off at the base and carried forward with the root-tip as the root elongated. There is a tube-like collar at the base of the adult root, which is the remnant of the torn-off outermost root-sheath (Fig. 4).

The initials of the root-hairs arise just behind the apex and under the innermost root-sheath (Figs. 26–27). As the root completes its development, the apical cell itself becomes divided in several parts, each one piliferous (Fig. 28). The mature root-hairs are 2–3 mm. long, with a nucleus near the tip. The tip is lined by granular contents. The root-hairs have a bulge at the base and a characteristic knee-like bend (Fig. 28). In a young root the tangential section of the root-hairs gives a remarkably regular pattern (Fig. 24).

The root is formed on a constant trimerous plan. The transverse section shows that the cells are in multiples of three. The endodermis cells are a multiple of three, so also the root-hairs themselves. The vascular structure is very simple as in the stem, there being only two or three spiral tracheidal elements. When a root is seen under the microscope the air

contained in the tracheids shows as dark lines, like the mercury line of a thermometer.

The mature roots are deciduous. After they attain a length of 40–50 mm. they drop off. The root-hairs provide accommodation for a large number of protozoa, algæ and soil particles. The roots contain some green pigment when young. The older roots look white. The deciduous roots come off with a neat end, owing to the presence of a basal absciss layer (Fig. 27) as in the case of the deciduous branch (Strasburger, 1873, Fig. 71, Pl. 5).

Sporocarps.

The sporangia are borne inside the so-called sporocarps, which are generally regarded as basipetal indusiate sori on the analogy of the Gradate ferns. The sporocarps arise from the ventral lobe of the first leaf of a branch, the dorsal lobe forming an involucre enveloping the sporocarps (Figs. 33, 40, 41). The sporocarps are borne in pairs, either both of the same sex, or one male and the other female (micro- and mega-sporocarps). At their base are found a number of filamentous hairs (Figs. 34, 41, 43). The sporocarps receive a vascular bundle which penetrates a short way up the stalk (Fig. 43).

The leaf-lobe, when its first median division occurs, at once begins to develop the two sporocarps. The two sporocarp initials grow by means of a three-sided apical cell for a short time. When three series of segments are cut off a ring-shaped projection arises about their base (Fig. 35). This is the beginning of the indusium (Figs. 37, 41, etc.). The apical cell of the sporocarp now cuts off an opercular cell and thus becomes a terminal mega-sporangium (Fig. 35). The ring-like outgrowth at its base becomes two-layered and grows up simultaneously with the sporocarp, so that by the time the primary tapetal cells are formed, the indusium stands as high as the sporangium. Now from the base of the sporangium arise all round a number of papillate outgrowths (Fig. 36), the cells containing dense proto-plasm like the tapetal cells and the central cell of the sporangium. These are the beginnings of the microsporangia. The sporangial capsule forms the one-layered tapetum and eight megaspore mother-cells. Shortly after the microsporangial protuberances appear, the walls of the tapetal cells of the megasporangium dissolve. The tapetal nuclei lie around the now separated and rounded off spore mother-cells (Figs. 36, 37).

The megaspore mother-cells divide and form eight tetrads. The in-dusium now grows up to enclose the megasporangium, all except at the top. *Anabæna* cells enter through this opening and lie on the top of the mega-sporangium (Figs. 39, 41, etc.). From this point onwards the two kinds of sporocarps begin to be differentiated.

Megasporocarp.

In the megasporangium, thirty-one of the megaspores abort, while only one (Figs. 38, 42), which usually holds the central position, continues to develop. The megasporangium increases in size. Its stalk ceases growth, as also the young microsporangia at the base of the megasporangium. As the latter grows in size, it completely fills the sporocarp and the microsporangia are squeezed down against the stalk until they become hardly recognizable.

The functional megaspore increases in size (Fig. 42). It is surrounded by densely granular protoplasm in which can be seen the tapetal nuclei. A beak-like projection forms on the top of the spore. This is the beginning of the conical body at the top of the spore, which gets surrounded by the float-corpuscles.

The ripe female sporocarp (the megasporocarp) is about 1·5 mm. × 1 mm. It is pointed at the tip. The cells of the upper half of the indusium become hardened, lignified, and dark-coloured, so that after the lower part decays, these upper, dark-coloured cells remain as a conical cap at the top of the spore, until it is pushed aside by the growth of the embryo (Figs. 58, 64, 66). The thickening of the cell-walls of the inner layer of the cap is peculiar (Fig.65). It takes the form of hair-like growths into the cavity of the cells.

A longitudinal section of the mature sporocarp (Fig. 45) shows that the spore with its float-corpuscles fills the sporangium completely; and that the latter is in close contact with the resting cells of *Anabæna*. The megasporangial wall is at this place represented by the membrane which bears numerous hairy appendages, and which becomes cup-shaped (Fig. 64) when the indusial cap is pulled off.

The ripe megaspore is more or less globular, with a firm yellowish exospore, which in sections is seen to be radially striated (Fig. 45). Outside this are two more epispore walls the middle one of which is granular in section, which at the top of the spore forms the spongy conical structure, which is surrounded by the float-corpuscles. The outermost epispore wall, which has a densely granular structure, bears curious, rigid, fluffy-looking bodies (Fig. 47), which later become rod-shaped (Figs. 48, 49). Thread-like outgrowths arise from the bases of these rods. (For the origin of these episporic appendages see the Discussion, p. 189.)

The megaspore is capped by the float-corpuscles, which form the "Swimming apparatus" of Strasburger (Strasburger, 1873, 66, and Campbell, 1893, 163). These consist of nine parts in three groups, *i.e.*, a ring of six below and of three above. The lower floats are slightly sunk in shallow

cavities in the sides of the central, spongy, conical structure. From the latter, as well as from the apex of the floats, filaments, like those growing from the papillæ on the surface of the spore, are produced in large numbers. The compartments of the floats which look like the cells of a honeycomb, are not cellular in nature. They arise by vacuolation in the protoplasm of the megasporangium. The similarity in structure between the massulæ and the epispore of the megaspore warrants the conclusion that the two are homologous. The threads attached to the epispore may "morphologically as well as physiologically be compared to the glochidia" (Campbell, 1893, 164).

Sometimes a cleft may be seen extending upward part way through the central conical mass, as Campbell (1893, 164) also states of *A. filiculoides.*

The megasporocarps become ripe by about April and are liberated from the mother plants. They float about for some days and then sink to the bottom. At this stage the indusial wall disorganises, all but the cap. The megasporocarps remain at the bottom till September.

Microsporocarp.

We have now to trace the development of the microsporocarp from the stage at which the male and female sporocarps were yet undifferentiated. As above stated, a terminal megasporangium is developed in both the sporocarps. In the microsporocarp, however, this degenerates. This happens only after the tetrad divisions of the megaspore mother-cells have taken place. Thus there is no doubt that this is an abortive megasporangium, as Strasburger and Pfeiffer (1907) concluded from their study of *A. filiculoides.* Its contents shrivel up, to the form of a few granular condensations (Figs. 43, 57). This structure has been referred to by some authors as the columella (Pfeiffer, 1907, 451), but this is confusing because this term was originally applied by Strasburger to the elongated receptacle on which the microsporangia arise (Strasburger, 1889, 8, and Fig. 1 *ma*, Pl. 1).

As in the case of the megasporocarp, papillæ are formed on the stalk of the megasporangium at its base, the beginnings of the microsporangia. Their growth was arrested in the megasporocarp ; but in the microsporocarp, many more of these are formed in basipetal succession, and develop into the microsporangia.

A young microsporocarp, soon after it has begun to develop into one, can be distinguished as it is broader than the megasporocarp of about the same age. The *Anabœna* cells creep in at the top of the indusium in the usual way. The indusium is two layers thick as in the female sporocarp. Its tip is thickened and hard as in the female sporocarp, but here it is short and abrupt. The body of the mature sporocarp is globular, only slightly

losing its shape by being pressed against its fellow, during the increase in size of both. The microsporocarp is more than twice the size of the female (Fig. 46). Like the latter it receives a vascular bundle into its short stalk.

The microsporangium initial has dense contents. Its development proceeds in the typical leptosporangiate manner. The first division is oblique (Figs. 50–52), producing an apical cell which after forming two further cells becomes a three-sided apical cell. The next division is periclinal, cutting off an opercular cell enclosing the apical cell. The latter then cuts off a layer of tapetal cells (Fig. 52), and itself undergoes division till a 16-celled tissue is built up. These sixteen cells are the microspore mother-cells. They undergo tetrad-divisions; the tapetal cells lose their walls and their nuclei divide into a large number and help in the nutrition of the sporogenous cells.

In the meantime the sporangial stalk elongates. It is composed of a row of cells, two cells in thickness (Fig. 53). Branching of the microsporangial stalk is sometimes seen, the branch bearing either another microsporangium or a sterile cellular filament (Fig. 53), which might be regarded as a sterile microsporangium.

Sixty-four microspores are developed. They remain thin-walled, with very little of granular contents. A clear triradiate mark is visible on their surface. The ripe spore is about ·035 mm. in diameter.

A very large number of microsporangia are produced in the microsporocarp, a median section of a full-grown specimen showing as many as thirty.

When the spores are nearly mature the formation of the massulæ begins (Fig. 56). In each sporangium there are formed 3–6 of them. They arise by vacuolation of the hardened protoplasm, resulting in a foamy appearance. The tapetal nuclei lie around the massulæ, and they later develop—partly at least—the glochidiate processes on the inner sides of the massulæ, i.e., along the faces where adjacent massulæ are in contact, as shown by Strasburger (1889, 17) in A. filiculoides. The glochidia themselves partake of the vacuolate structure of the massulæ. They are of various shapes; some simple processes, others branched and hooked (Fig. 55).

The ripe microsporocarps are liberated from the mother plant. They are red when shed but they soon lose the colour. They float about for some time and then sink to the bottom. The wall disorganises, and the bunches of microsporangia can be seen for a long time, at the bottom of the water. Later the sporangia get detached, their walls also disorganise and the massulæ containing the spores are set free. Massulæ were found both

floating and sunken in September. The floating ones examined did not show the germination of the microspores. Some of them, however, were quite empty of spores, indicating a liberation of antheridia.

Germination of the megaspores.

The megasporocarps remain at the bottom of the ponds during the summer. They germinate after the rains.

As stated above, the indusium of the sporocarp disorganises all but the thickened tip which remains as a mitre on the top of the float-corpuscles.

The ripe megasporocarp is full of oily contents which escape as a cloudy mass when a sporocarp is punctured under water. The oil, no doubt, is the food reserve.

I have not seen how the first divisions take place in the development of the female prothallus. From sections examined of the mature prothallus it is found that it fills only the upper part of the spore-cavity. Berggren's observations (Berggren, Pl. 1., Figs. 13, 15, 16, 19 and 21) on *A. caroliniana*, and Campbell's (1893, Pl. 8, Figs. 43–49) on *A. filiculoides* show that in both these species the condition is as here described in *A. pinnata.* Mr. Sud's (1934, 195, Fig. 9) statement that in *A. pinnata* the prothallium fills the whole cavity of the megaspore is incorrect. I have not been able to ascertain when the germination of the megaspore begins : whether before the detached sporocarps sink to the bottom or after.

When the prothallus is fully developed and the archegonia are formed, the sporocarp floats up to the surface. The enlarging prothallus makes its way through the tri-radiate opening at the top of the spore. It pushes aside the floats and thus all the nine of them become separated from one another and project outwards, the lower six forming a ring below (Figs. 58-62, 64) showing six floats symmetrically placed, and the upper three in a ring above. The indusial cap is still at the top in an erect position.

I put some sporocarps in Knop's solution* for algal culture, together with some microsporangia and massulæ. The sporocarps floated up to the surface after about fourteen days. And within three days of this the young embryo plants had already developed in the same dishes.

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* KNOP'S SOLUTION.

1.	Magnesium sulphate	..	0.25	gm.
2.	Calcium nitrate	..	1.00	gm.
3.	Potassium phosphate	..	0.25	gm.
4.	Potassium chloride	..	0.12	gm.
5.	Ferric chloride	..	1	drop
6.	Water	..	1,000	c.c.

A large number of archegonia are produced in *A. pinnata* (Fig. 73), unlike in *A. filiculoides* (Campbell, 1893, Figs. 49 51, 54, 55, 60 and 61), or *A. caroliniana* (Berggren, Pl. 1, Figs. 4-6, 10, 11, 13 and 15). More than thirty were noted in one case (Fig. 73). The oldest archegonia are at the centre, where the prothallus is somewhat depressed. The venter is rather large, and adjacent archegonia are often separated by only a single layer of cells (Fig. 63). The neck is formed of two rings of four cells each which are radially seriate (Fig. 73) so that when seen from above they look like two concentric circles traversed by a cross. The contents of the archegonia were not preserved in all the preparations. Only one of them seems to show a fertilisation stage (Fig. 76).

The prothallial cells are thin-walled and contain chlorophyll (Fig. 67).

Male Prothallus.

I have not been able to follow the germination of the microspores. Mature male prothalli, however, are seen in large numbers in sections of the female prothallus developing the young embryo. The male prothalli were lying freely on the surface of the female prothallus, in the neighbourhood of the archegonia (Fig. 68).

An important point which my investigations have brought out is about the male prothallus. In *A. pinnata* the prothalli escape from the massulae which get hooked on to the megasporocarp (Fig. 64), and come to lie about the female prothallus. Campbell (1893, 169) who studied the germination of the microspore and the development of the antheridia, did not see the dehiscence of the antheridia. He says that the ripe prothallium remains completely embedded in the substance of the massulae, and conjectures that probably the spermatozoids escape by a softening of the outer surface of the massula. Judging from the case in *Salvinia*, we should expect that in *Azolla* also the male prothalli escape as a whole from the massulae as they do in *Salvinia*, where they are said to be easily detachable (Campbell, 1928, 400). The facts observed in *A. pinnata* fulfil this anticipation and suggest that the same is the case in all the other species of *Azolla*.

Fertilisation.

Fertilisation most probably takes place at the surface, after the female sporocarp has floated up. The archegonia of the prothallus which just came up to the surface showed no fertilisation. Campbell (1928, 414) says the term "Swimming apparatus" applied by Strasburger to the float-mass is a misnomer, as the sporocarps sink to the bottom when they are shed from the mother plants. If this were true, it would be hard to explain their presence. I think that the term is still applicable, but that the floats become really

functional when the female prothallus has developed and the archegonia are ready for fertilisation.

Embryo.

I could not follow the development of the embryo plant in its early stages for lack of material. As the embryo develops it pushes aside the indusial cap, which at this stage is thus tilted at 90° (Figs. 58 62, 64, 66). The megasporocarp remains attached to the young plant till it has produced 8–10 leaves. The "foot" remains embedded in the archegonium. The lower walls of the cells of the foot, *i.e.*, where it is in contact with the floor of the archegonium, becomes somewhat thickened (Fig. 70).

The development of the stem-tip of the embryo plant is similar to that of the adult plant. I could not study the development of the first leaf or "cotyledon". The first root of the embryo grows obliquely on one side (Fig. 74). The lateral branches are formed by the young plant before it has produced 8 or 10 leaves.

The resting cells of *Anabæna* present at the tip of the indusium get into the cavities of all the leaves, except the cotyledon. As Campbell describes (Campbell, 1893, 182) "they assume the blue-green colour of the active cells, elongate and divide rapidly by a series of transverse walls into short filaments that at first look like *Oscillaria*". The cells are then rounded off, the heterocysts are formed and the typical form of the ordinary filaments is attained.

Discussion.

In his preliminary note on *A. pinnata* Sud (1934, 190) says that his observation about branching of the stem differs from that given by Engler and Prantl (1902, 1 Teil, 4 Abt., 401). He has mistaken the term "extra-axillary " and says "that is, here, a lateral branch is said to rise opposite a leaf". The term only means that branching is not in relation to the axil of a leaf, as Sud himself has observed (Sud, 1934, 190, Fig. 1). He also says that the growth of the leaf, at least in the earlier stages, is by means of an apical cell. I have been able to confirm Strasburger's (1873, 41) statement that there is no apical cell in the leaf of *Azolla*. The growth is by means of divisions of the marginal cells of the leaf, a kind of growth seen also in the development of the indusial wall. In a section of a young leaf, one of these marginal cells is necessarily found at either end of a section (Sud's Fig. 5*b*) ; these marginal cells Sud has mistaken for apical cells.

Sud's drawing of the female prothallus is incorrect. He says that the prothallial cells fill the whole of the spore-cavity (Sud, 1934, 195, Fig. 9) : "in the lower part the cells are smaller in size and in the upper they become

elongated and narrow. The latter are similar to the prothallus cells of *A. caroliniana* figured by Berggren ". The fact is that the upper part alone is the true prothallus. The lower part, as his drawing shows, is not cellular tissue at all. He has mistaken the granular condensations of protoplasm and food matter for cells.

Sud did not observe massulæ attached to the megasporangia (Sud, 1934, 195) and conjectures that the glochidia are rudimentary and do not serve for attachment. My observation shows (Fig. 64) that the massulæ get attached to the megasporangia as is known in *A. filiculoides* (Campbell, 1893, Fig. 75, Pl. IX) and *A. caroliniana* (Berggren, Fig. 2, Pl. 1).

The stoma of the mature leaf in *A. pinnata* is a simple perforate cell. In *A. filiculoides* Strasburger has shown two septa across the guard-cells. Haberlandt (1914, 469) says that in *A. caroliniana* also the 'pore is elongated at right angles to the plane of the septa between the two guard-cells and these septa finally become partially or entirely obliterated'. I could not see any such septa in my preparations of *A. pinnata*. It may be that I did not come across young enough stages.

The constant association of *Anabæna* with *Azolla* is interesting. I have never seen a leaf-cavity or a megasporocarp of *Azolla* which does not contain *Anabæna*. But the physiological aspect of the relation between the two plants needs critical investigation. Neither of the plants seems to suffer by this association. Whether they are mutually beneficial is not known. Judging by the presence of *Anabæna* about the extreme apex of the stem and of an early development of an invagination in the dorsal lobe of the leaf, it would seem as if the *Anabæna* induces a stimulus to the formation of the cavity. The *Anabæna* has become closely adapted in conformity with the life-history of *Azolla*; the cells entering the sporocar)s become resting cells just as the sporocarps are also resting organs. The resting cells of the *Anabæna* germinate when the embryo plant develops.

The origin of the involucre enveloping the sporocarps has been a subject of controversy. Strasburger held that the sori represent the transformed leaf-lobes, and conceived the involucre as the lower lobe of a leaf, while Campbell came to the conclusion that the whole of the ventral lobe goes to form the sori and that the involucre is derived from the whole of the dorsal lobe. Goebel (1918, 1133), who examined this point again, says that the sporophyll results from a division of the lower lobe, which occurs early : the whole of the upper lobe does not form the involucre, but remains as before, receives a vascular strand, and possesses an *Anabæna* cavity ; and at its base it produces a wing-like flap which envelopes the sori. My observations confirm those of Goebel (Fig. 40).

Strasburger calls only the outermost sheath around the root as the root-sheath (Strasburger, 1873, 45) while the two inner he calls the root-cap proper. In all typical roots the root-hairs arise behind the root-cap. In *Azolla* the so-called root-cap envelopes the whole root, root-hairs and all. I think it would be more appropriate to call all the three layers by the name root-sheaths. The root-sheath is a peculiar analogue of the root-cap, but is not a true root-cap. The older workers called the two inner sheaths a root-cap proper and the outermost a root-sheath as if for fear of saying that there is no root-cap developed in *Azolla*, while they were aware that the whole structure was unique.

The appearance of a primary megasporangium in both mega- and microsporocarps shows that perhaps originally the sporocarps were bisexual. The degenerate megasporangium in the microsporocarp also indicates that the sporangial primordia about the megasporangium are microsporangia (Pfeiffer, 1907, 451).

The microsporangial stalk is seen branching in some cases, the branch bearing either another microsporangium or a sterile cellular filament which may be considered an abortive sporangium. This occasional feature recalls the branched sporangial stalks of *Salvinia*.

There is no trace of an annulus in the sporangia. This is perhaps to be attributed to the aquatic habit of the plant. This is the case in all the Hydropterideæ.

In the megasporangium the spore embedded in the epispore and capped by the spongy conical body, and the float-corpuscles are to be homologised to the massular divisions in the microsporangium. The conical body may be regarded as the homologue of the tri-radiate mark. As Campbell (1893, 164) says "the threads attached to the epispore may morphologically as well as physiologically be compared to the golchidia". The spore capped by the conical structure and surrounded by the epispore is to be regarded as the single functional spore of the single fertile massula. The floats may be regarded as sterile massulæ.

Strasburger and Campbell agree that the episporic thread-like appendages are formed by the tapetal nuclei and protoplasm. A reference to our Fig. 65 showing the peculiar thread-like thickenings arising from the cell-walls of the indusial cap suggests that the appendages have perhaps partly at least been contributed by the thickening in the megasporangial wall.

Several botanists have suggested an analogy between the megasporocarp of *Azolla* and the seed of the spermaphytes. Eichler (in Engler and Prantl, 1889, Teil 2, Abt. 1, p. 16) has used the term "monangic sorus" for an ovule

and regards the integument as homologous with the indusium. The seed-like modification of the megasporocarp is a much more modern contrivance than the seed (Arber, 1906, 229).

The present study has shown that while on the whole the structure and life-history of *A. pinnata* are very similar to those of the other species described, there are important differences in detail.

The genus *Azolla* holds a somewhat isolated position. Its nearest ally is unquestionably *Salvinia* with which it is placed in the family Salviniaceæ. The Salviniaceæ have their affinities with the homosporous Filices, or the lower members of the leptosporangiate series, and probably with the Hymeno-phyllaceæ--Gradate Marginales of Bower (1928, Vol. 3, p. 262). The Salvi-niaceæ and Marsileaceæ, both heterosporous Leptosporangiatæ, have had a special line of development, differing from that of the other Filices, as a consequence of their aquatic or amphibious habitat.

Fossil History of the Hydropterideæ.

It may not be out of place here to give a sketch of the fossil history of the Hydropterideæ. It will be of interest on account of the extreme specialisation of the group.

Salviniaceæ.--Salvinia is represented by several Tertiary species. Florin (1919) has given a list of ten such species, nine of them ranging from Eocene to Mio-Pliocene, and one from what may be the Upper Cretaceous rocks of Carbonado, Washington (Seward, 1910, Vol. 2, p. 262). There are several others in addition to these (Berry, 1930, Kirchheimer, 1929, 1930, 1931 and 1932). The species for the most part have been founded on leaves only, a few on sporocarps alone, and a very few on complete specimens.

Three extinct species of *Azolla* have been described, *A. intertrappea* Sahni and Rao (Sahni and Rao, 1931, Part 4, pp. 26 27) from the Deccan Intertrappeans of India, *A. tertiaria* Berry (1927, 4 5) from Western Nevada and *A. prisca* Reid and Chandler (1926, 407), from the Oligocene of the Isle of Wight. Both *A. pinnata* and *A. filiculoides* have been recorded in the fossil state from Pleistocene deposits in Holland (Florschütz, 1928). These two present-day species have not been found in rocks earlier than the Pleistocene. The Indian species *A. intertrappea*, which as Sahni (1934 *a*, 134–136) has shown is probably of Eocene age and therefore the oldest known species of the genus, shows characters resembling the species assembled under Euazolla, *viz.*, in having three "floats" and anchor-like glochidia. This supports the view above expressed that the present-day Indian species, *A. pinnata* has been derived from the Tertiary species which resembles *A. filiculoides*. Also, the nine-floated "swimming apparatus" seems to be more

recent than the three-floated condition. *A. prisca* Reid and Chandler (1926) combines the characters of the two sections of *Azolla*, Euazolla and Rhizosperma. The occurrence of glochidia and the tubercled filament-bearing macrospore shows that the fossil is related to the Euazollæ—more particularly to the species *A. filiculoides*. But in the presence of nine floats to the macrospore it resembles the Rhizospermæ. Probably, therefore, it represents an ancestral type in which features now distributed were combined. Reid and Chandler, however, say that it must belong to an earlier stage of evolution than the two sections.

As regards the Palæozoic genera *Traquairia* Carr. (Mrs. Scott, 1911, 459-467, Pls. 39-40) and *Sporocarpon* Will., Prof. Seward says that comparisons have been made with the reproductive organs of *Azolla*, but that these rest on a wholly insufficient basis (Seward, 1910, Vol. II, 476). Similarly in the case of *Protosalvinia* Dawson and *Chorionopteris* Corda a relation with the Hydropterideæ has been suggested on insufficient evidence (Seward, 1910, Vol. II, 476).

Marsiliaceæ.—The fossil history of the Marsiliaceæ is more uncertain. The Wealden genus *Marsilidium* Schenk (Seward, 1910, Vol. II, 474) cannot be regarded as satisfactory evidence of the family in the early Cretaceous flora. The Cretaceous *Marsilia Andersoni* Hollick (Seward, 1910, Vol. II, 474) is too fragmentary to be accorded that generic name. The fragment figured by Heer from the Tertiary rocks of Oeningen as *Pilularia pedunculata* is too small to determine with reasonable accuracy (Seward, 1910, Vol. II, 475). A few other fossils are still more doubtfully assigned to *Marsilia*.

The Mesozoic genus *Sagenopteris* seems to have had a long-standing claim to be compared with *Marsilia*. It has, however, recently been shown by Hamshaw Thomas (1925) that *Sagenopteris* leaves are in all probability the foliage of plants which bore reproductive organs indicating affinity to the *Caytoniales* (Seward, 1931, 309, 367, etc.).

According to Harris (1931, 139) "The small ovoid fossil *Hydropterid-angium marsilioides* Halle, which Halle suggested was a sporocarp of the Marsiliaceæ, is altogether doubtful. Its structure is inconclusive and the associated *Marsilia*-like leaves—*Sagenopteris*—are now thought to belong to the Caytoniales". From his own study of better preserved Rhætic material from East Greenland Harris (1932, 122-127, Pl. 9, Pl. 10, Figs. 3 8, Pl. 11, Figs. 1, 2, 15; Text-Fig. 52) concludes that the relationship between *Hydro-pteridangium* and the Hydropterideæ is out of the question. He says that it must belong to a seed plant (Gymnosperm) and its spores must be microspores.

The families Salviniaceæ and Marsiliaceæ are probably not at all related together. The latter seems to have affinities with the Schizæaceæ, while the former is perhaps nearest related to the Hymenophyllaceæ (Bower, 1908, 551 ; and 1928, Vol. III, 262). They are generally grouped together on account of their heterospory and aquatic habit.

We may conclude this brief summary with the authoritative opinion of Professor Seward : "There is no evidence contributed by fossil records which indicates a high antiquity for the Hydropterideæ. It is unsafe to base any conclusions on the absence of any undoubted palæozoic representatives of this group ; but the almost complete absence of records in pre-Tertiary strata is a fact which may be allowed some weight in regard to the possible evolution of the heterosporous filicales at a comparatively late period in the earth's history" (Seward, 1910, Vol. II, 477).

Summary.

Work on *Azolla pinnata* was taken up as a necessity was felt for a comprehensive account of at least one species other than the well-known *A. filiculoides*. *A. pinnata* seemed specially suitable as it belongs to the second sub-genus Rhizosperma.

A short historical review of the previous work is given.

In the structure and development of stem, leaf, root, sporocarps, gameto-phytes, etc., *A. pinnata* is essentially similar to the other well-known species, while there are important differences in detail. The general course of the life-history is as follows : fertilisation takes place in September or October. The resulting fresh plants mature in Spring. By about April the sporocarps ripen, and are shed. The spores rest during Summer. The megasporocarp with the attached massulæ floats up before fertilisation.

The Discussion takes notice of the incorrect observations of Sud and other workers. Goebel's view of the nature of the involucre has been con-firmed. The advisability of employing the term root-sheaths, instead of both root-sheaths and root-cap is suggested. The author has observed an occasional branching of the microsporangial stalk, bearing more sporangia than one, or the sporangia represented by sterile filaments. A peculiar mode of thickening in the cells of the indusial cap is noticed.

An important point brought out is that the male prothalli of *A. pinnata* are detachable. They escape from the massulæ which get hooked on to the megasporocarp, and come to lie about the female prothalli. It is suspected that the same occurs in the other species.

A brief summary of the fossil history of the Hydropterideæ is given. Literature on the fossil species of *Azolla* shows that the *A. filiculoides* type

(*viz., A. intertrappea*) is the oldest known, *viz.,* in the Eocene; a synthetic type combining the characters of the two sub-genera is found in the Oligocene, and both *A. filiculoides* and *A. pinnata* are known from the Pleistocene. There is no evidence in the fossil records for a high antiquity for the Hydropterideæ : the oldest authentic records are Tertiary.

Further work on the germination of the spores and early development is hoped to be taken up.

Acknowledgments.

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EXPLANATIONS OF PLATES.

ABBREVIATIONS USED.

a	..	Point of attachment.
ab	..	Absciss layer.
a.mg.	..	Abortive megasporangium.
ar	..	Archegonium.
c	..	Collar around base of root.
d.l.	..	Dorsal lobe of leaf.
e	..	Endodermis.
fl	..	Float.
f.m.	..	Fragment of megaspore wall.
h	..	Hair.
h.a.	..	Hairy appendages.

i.c.	..	Indusial cap.
in	..	Involucre.
m	..	Megaspore.
ma	..	Massula.
mg.w.	..	Megasporangial wall.
mi	..	Microsporangium.
m.p.	..	Male Prothallus.
o.a.c.	..	Opening of *Anabæna* cavity.
p.	..	Pericycle.
ph	..	Phloem.
r	..	Root.
r.h.	..	Root-hair.
r.s.	..	Root-sheath.
s	..	Stoma.
st	..	Stem.
th	..	Thickening.
t.n.	..	Tapetal nucleus.
v.l.	..	Ventral lobe of leaf.
x	..	Xylem.

PLATE XIII.

FIG. 1.- A sporophyte plant of *Azolla pinnata*, showing the root with the characteristic root-hairs. × 6.

FIG. 2 —Plant seen from above. × 6.

FIG. 3- Plant seen from below showing the sporocarps. Their involucres are shown black. × 6.

FIG. 4.—Plant seen from below. Note the collar around the base of the root. × 6.

FIGS. 5 & 6. Stem apex, with the beginnings of the lateral organs. × 300. See also Fig. 8.

FIG. 7 -A young leaf-lobe showing the regular tangential and radial cell-divisions. × 480.

FIG. 8 Stem apex. × 266. See (5) and (6).

FIG. 9 —Shows the margin of the lower leaf-lobe. × 80.

FIG. 10- Stele of the stem, slightly oblique. × 480.

FIG. 11 – Sagittal section through the apex of the stem showing the first division in the apical cell and an young root with three root-sheaths. × 480.

FIG. 12.—T.S. of part of the stem, showing stomata and hairs. × 300.

PLATE XIV.

FIG. 13.—A leaf. The underside of the dorsal lobe is seen with the opening of the *Anabæna*-cavity, with radiating cells around it. Note the stomata. The shaded part is thick. The clear margin of 4-5 cells is one-cell thick. × 48.

FIG. 14.- Section of an young dorsal-lobe of leaf showing the already formed *Anabæna*-cavity. × 123.

FIG. 15.—Section of a stoma of leaf. × 480.

FIGS. 16-19. –Hairs in the *Anabœna*-cavity of leaf. $\times$ 300.

FIG. 20.—-Absciss-layer of branch of stem. $\times$ 80.

FIG. 21. --Showing the dorsal and ventral leaf lobes, the *Anabœna*-cavity in the former and a simple cavity in the latter. Note the mouth of the *Anabœna*-cavity. $\times$ 123.

PLATE XV.

FIG. 22. -See Fig. 21. $\times$ 80.

FIG. 23.- L.S. of root. Note the three root-sheaths and the apical cell. $\times$ 266.

FIG. 24.- Tangential section of root, across the root-hair papillæ. The three root-sheaths are indicated. Note the regular pattern of the section. $\times$ 266.

FIG. 25. L.S. of leaf to show the *Anabœna*-cavity and its opening. $\times$ 300.

FIG. 26. Young root-hairs, with the intervening cells. The latter increase in number during the growth of the root. $\times$ 300.

FIG. 27. L.S. of part of root. Note the three root-sheaths, the absciss layer of smaller cells at the base of the root, and the tracheid from the stem, supplying the root. $\times$ 300.

PLATE XVI.

FIG. 28.--Tip of root after the root-sheath has been slipped off. $\times$ 80.

FIG. 29.- L.S. of young root, showing the four root-sheaths of which the second from outside is disorganising. $\times$ 123.

FIG. 30. Root-sheath slipped off from the adult root, the tip of which is figured in Fig. 29. $\times$ 80.

FIG. 31. -L.S. of young root, a stage later than the one seen in Fig. 29. $\times$ 300.

FIG. 32. -T.S. of root. $\times$ 480.

PLATE XVII.

FIG. 33. --L.S. of a pair of very young sporocarps, with their involucre arching over them. $\times$ 266.

FIG. 34.- Hairs at the base of the sporocarps. $\times$ 266.

FIG. 35. --L.S. of very young sporocarps. $\times$ 266.

FIG. 36. L.S. of young megasporocarp, showing the separating spore tetrads. Note the papillæ at the base of the megasporangium. $\times$ 300.

FIG. 37. --L.S. of megasporocarp, showing tetrad formation in megasporangium. $\times$ 300.

FIG. 38. --An oblique section of an young megasporocarp showing the single megaspore developing and three disorgamsing spores. The smaller nuclei are tapetal. $\times$ 300.

FIG. 39. L.S. of megasporocarp at an earlier stage than Fig. 38. $\times$ 300.

FIG. 40.- Shows the flap of the dorsal lobe of leaf which forms the involucre of the sporocarps. $\times$ 106.

PLATE XVIII.

FIG. 41.--L.S. of young megasporocarps showing the divisions in the sporogenous tissue. The tapetum is partly disorganising. $\times$ 433.

FIG. 42. L.S. of young megasporocarp showing the functional megaspore with a beak on its top. It is the beginning of the conical body at the top of the spore which gets surrounded by the float-mass. $\times$ 300.

Fig. 43.- L.S. of an young microsporocarp with the terminal abortive megasporangium. $\times$ 300.

Fig. 44. –L.S. of megasporocarp showing the megaspores and tapetal nuclei. From this stage the single functional megaspore develops further and the others abort. $\times$ 480.

PLATE XIX.

Fig. 45.- L.S. of mature megasporocarp partly reconstructed. The innermost spore-wall has broken and curled inwards. $\times$ 106.

Fig. 46.—Involucre surrounding the sporocarps. The microsporocarp (A) has been detached, its point of attachment being seen at 'a'. $\times$ 10.

Figs. 47–49.- Processes on the surface of the megaspore. Fig. 47 shows an earlier stage than Figs. 48–49. In Fig. 48 are seen the points of attachment of the thread-like appendages. Fig. 47 $\times$ 480; Fig. 49. $\times$ 300.

Figs. 50-52. -Three stages in the development of the microsporangium. $\times$ 480.

Fig. 53.—Stalk of microsporangium, two-cells thick. Note the sterile filamentous branch. Sometimes another microsporangium replaces the sterile filament. $\times$ 80.

Fig. 54.—Stellate hairs which form the wall of the microsporangium. $\times$ 80.

Fig. 55.- A glochidium. Note its vacuolated nature. $\times$ 480.

Fig. 56.—A massula with microspores and glochidia. $\times$ 80.

Fig. 57.---An oblique section of a microsporocarp with the terminal abortive megasporangium. $\times$ 253.

PLATE XX.

Fig. 58. --An young embryo plant, showing the two rings of floats, the female prothallus, the first leaf and the indusial cap tilted at right angles. See also Fig. 66. Diagrammatic.

Figs. 59–62. Young embryo plants with a few leaves only developed.

Fig. 63. T.S. of female prothallus showing nine archegonia, adjacent ones often separated by only one layer of cells. $\times$ 300.

Fig. 64.- Sporocarp with female prothallus. Note the massulae (shaded) attached to the sporocarp. There are seven of them. The floats are not shaded. See the cup-like structure at the top of the sporocarp from which a number of thread-like appendages arise. The indusial cap is tilted on one side; a few of its cells are shown rather diagrammatic. $\times$ 80.

Fig. 65. L.S. of the indusial cap. Note the peculiar thickening of the cell-wall projecting into the cavity of the cells, the resting *Anabæna* cells and the mass of thread-like appendages. $\times$ 48.

Fig. 66.—The embryo plant shown in figure 58 seen from above. Note the two rings of floats and the indusial cap. The margin of the cotyledon is shown black. Diagrammatic.

PLATE XXI.

Fig. 67.---L.S. of female prothallus showing two archegonia. The prothallial cells contain chloroplasts. $\times$ 48.

Fig. 68.—A male prothallus. $\times$ 1000.

Fig. 69. --T.S. of sporocarp with the female prothallus cut near its base. As the prothallus arches over the cavity of the megaspore, at this level, a section shows a clear

space in the middle. The six lower floats are arranged symmetrically all round. Note the hairy appendages and part of the megaspore-wall (black). $\times$ 123.

FIG. 70.—L.S. of embryo plant embedded in the female prothallus. The wall of the 'foot' cells in contact with the base of the archegonium is somewhat thickened (black). $\times$ 80.

PLATE XXII.

FIG. 71.—Photograph of *Azolla* plants closely covering the surface of the water. Nat. size.

FIG. 72. -Photograph of a megasporocarp with the prothallus developed. Seven massulæ are found hooked on to the sporocarp. See also Fig. 64.

FIG. 73.—Photograph of the female prothallus seen from above, showing more than thirty archegonia.

FIG. 74.—Photograph of a number of embryo plants at various stages of development. Note the obliquely borne roots of the older plants.

FIG. 75.—T.S. of the female prothallus showing a number of archegonia and a few male prothalli.

FIG. 76.—L.S. of the female prothallus showing an archegonium at the fertilization stage. Note the two nuclei.

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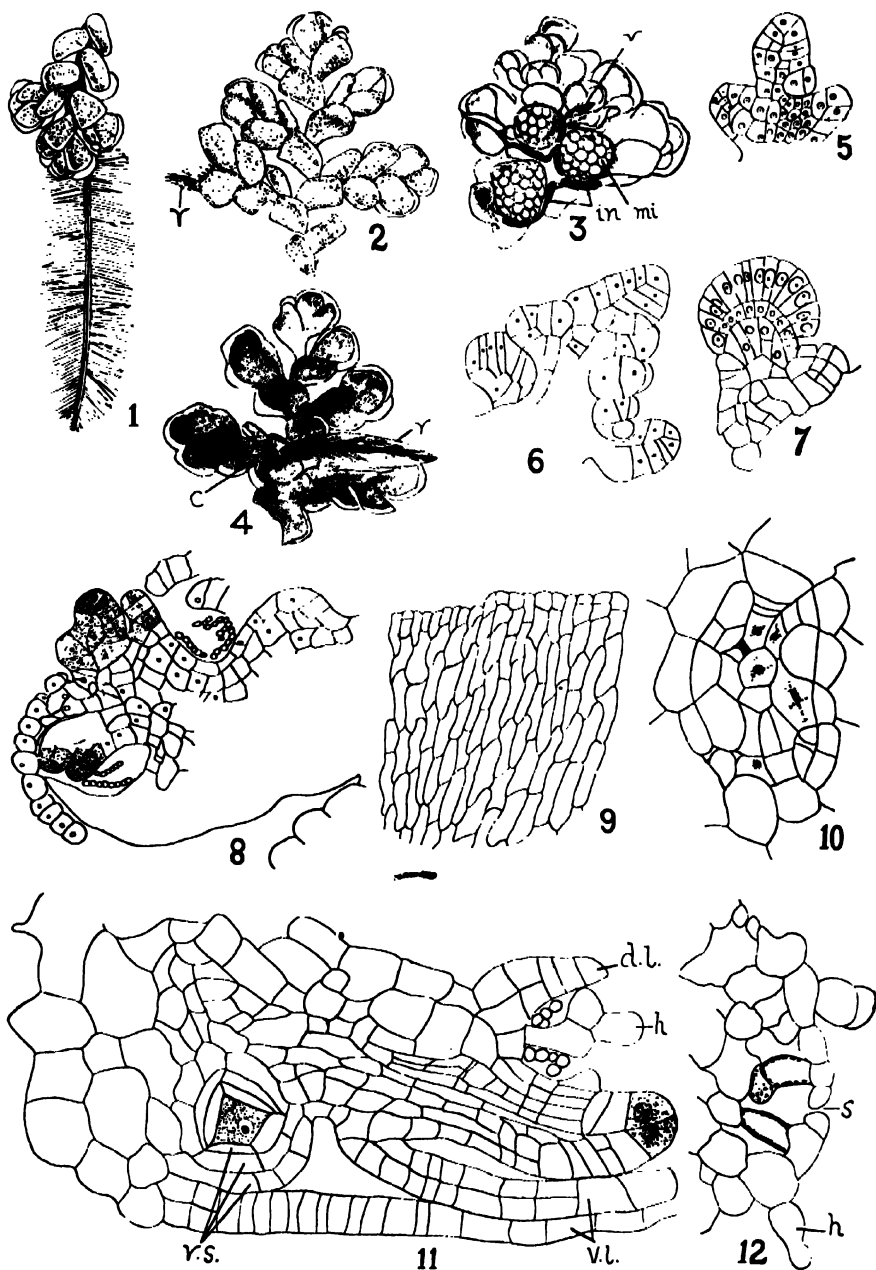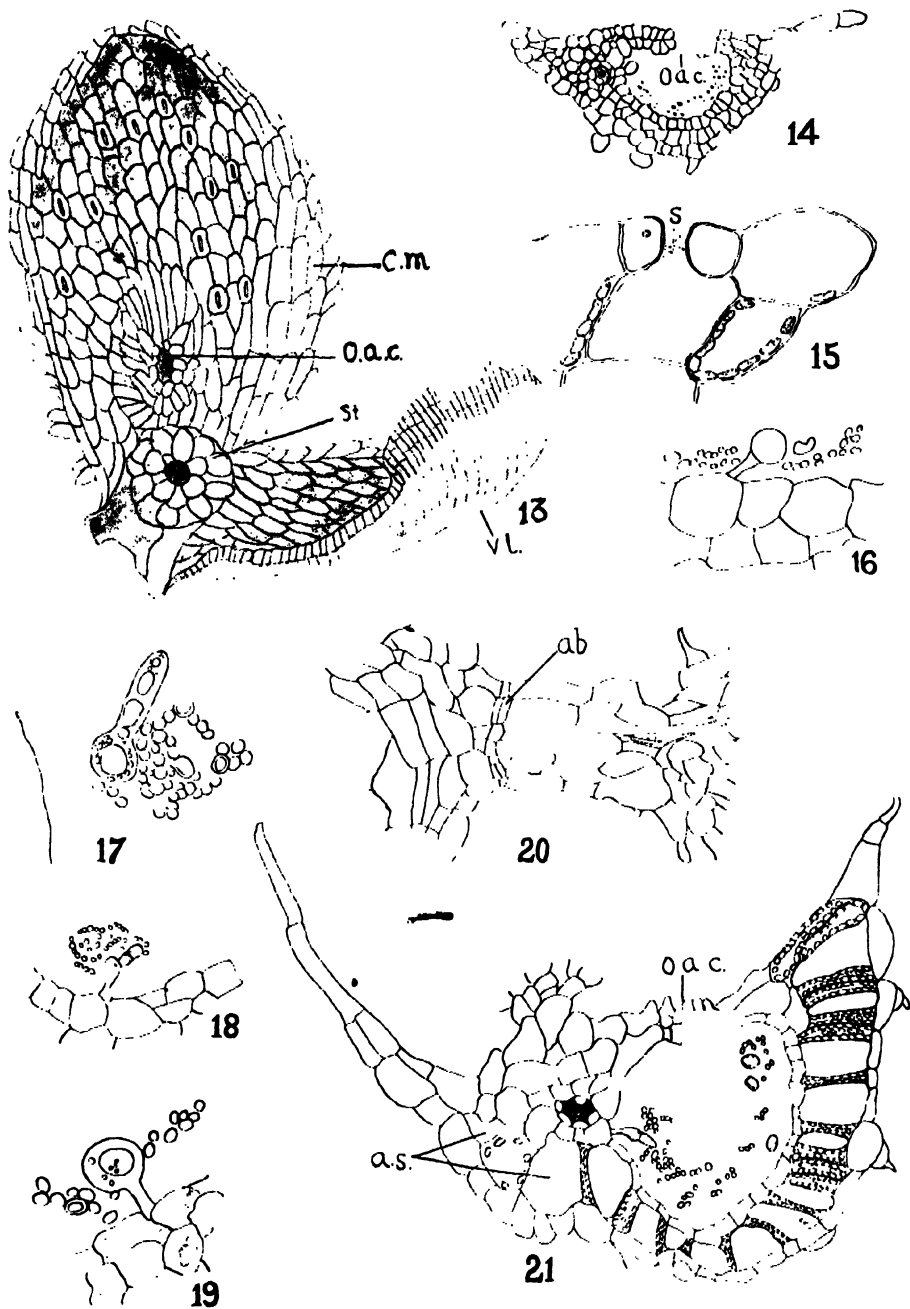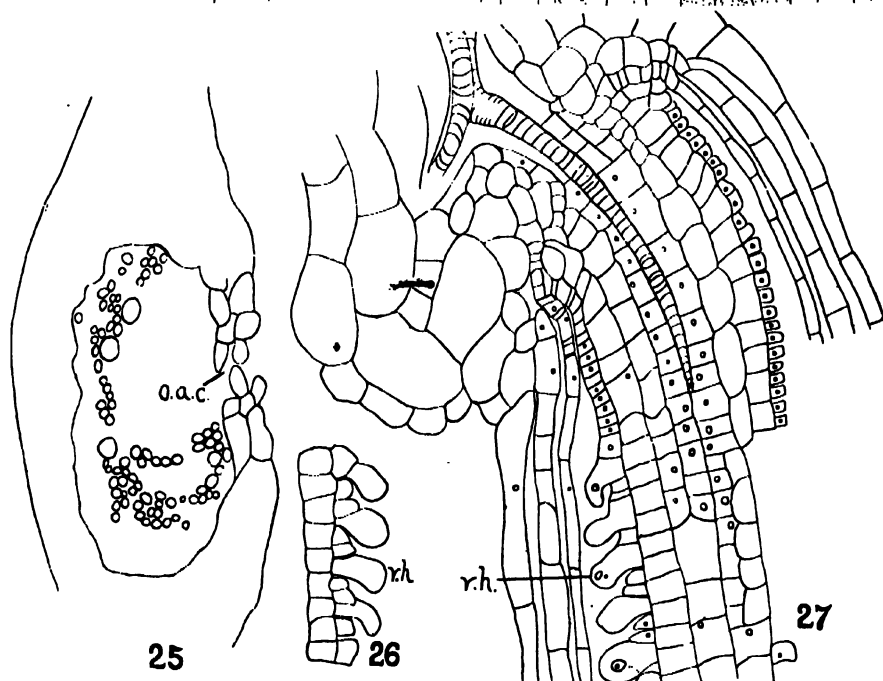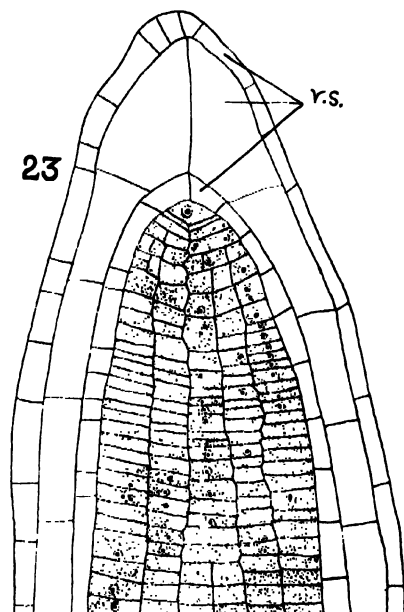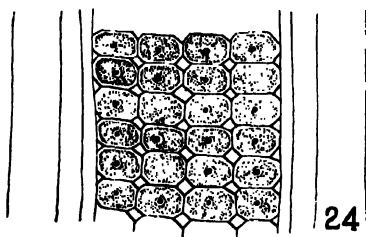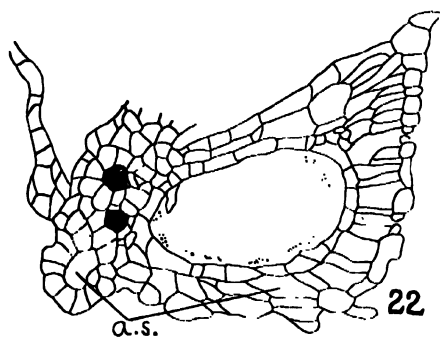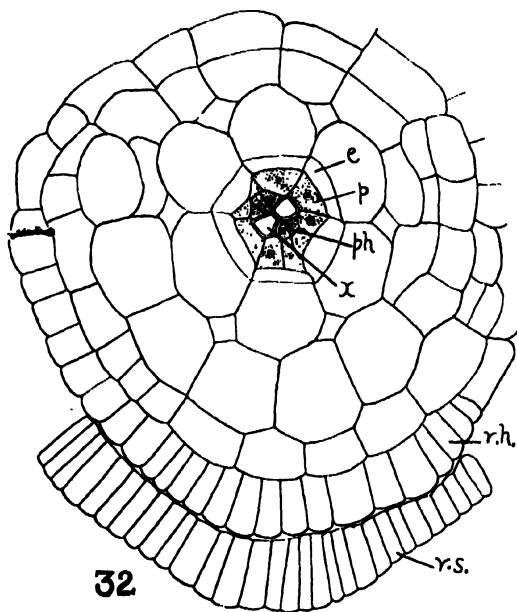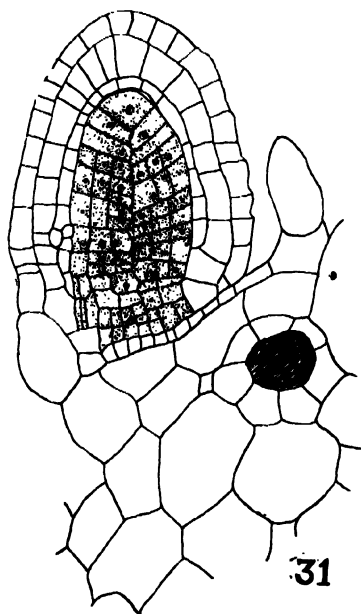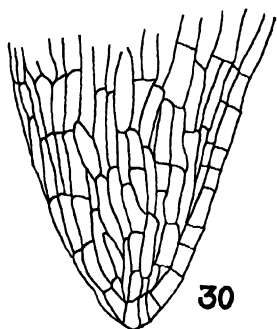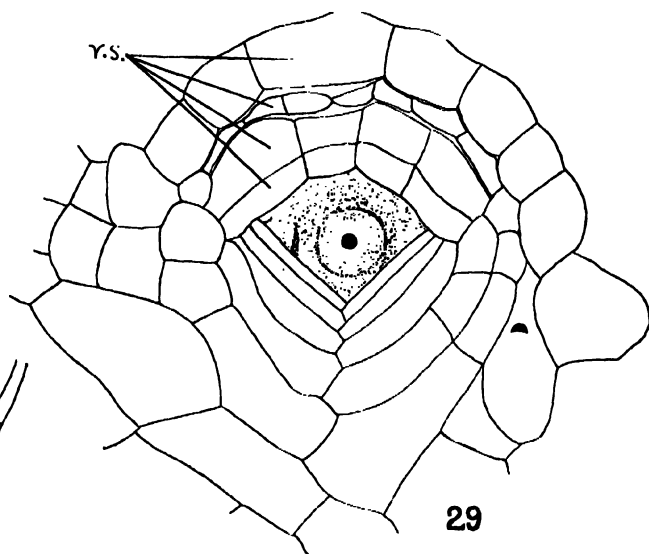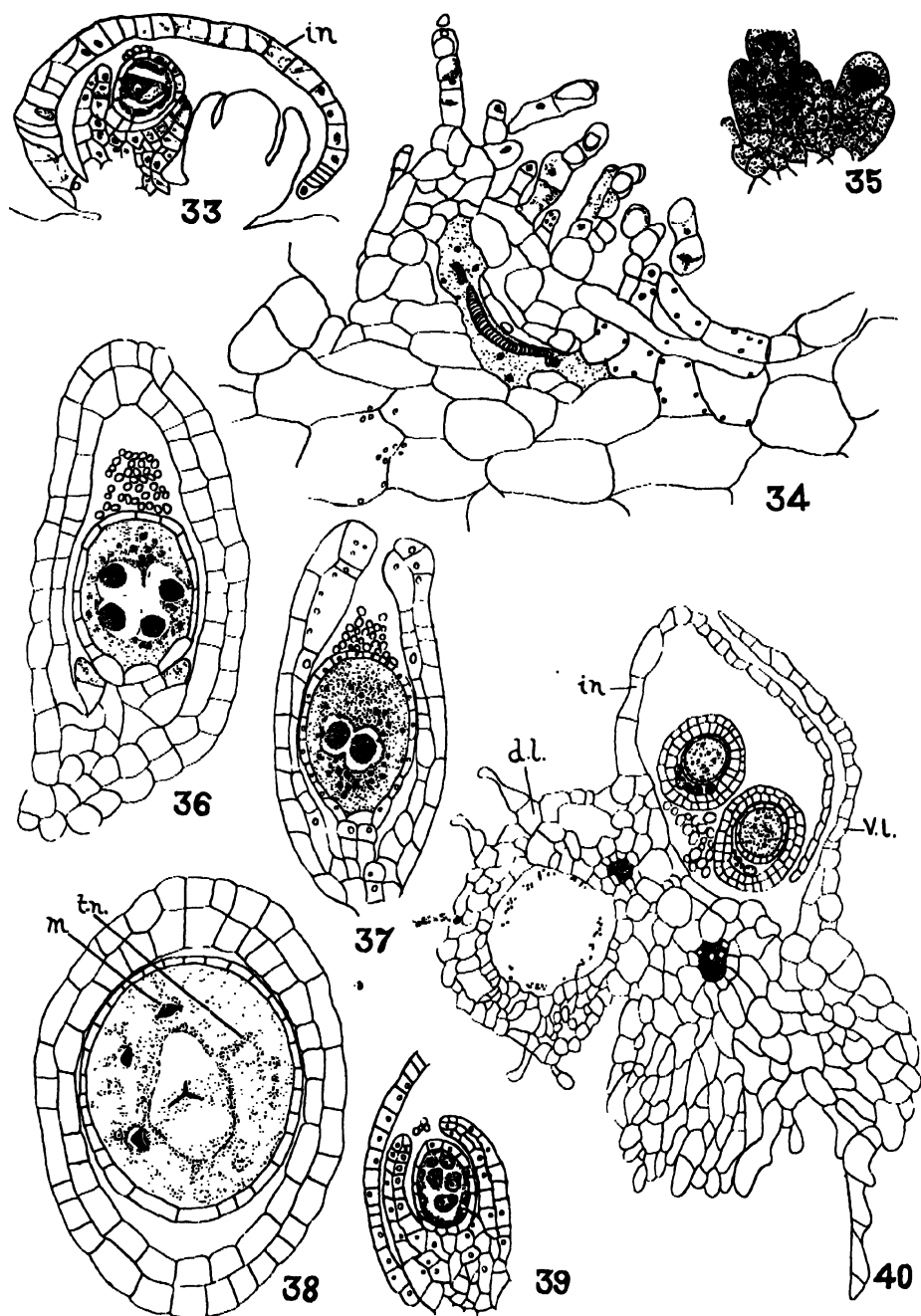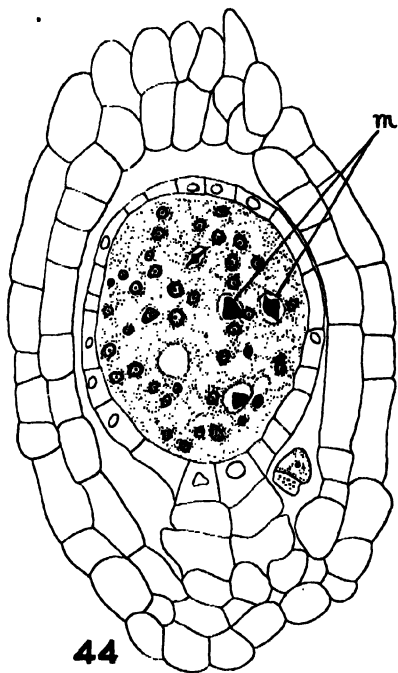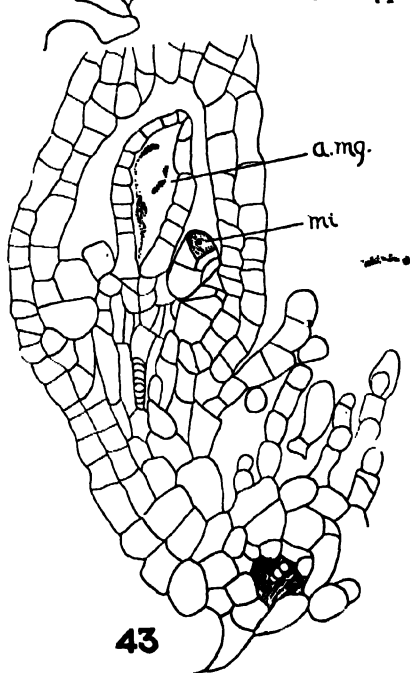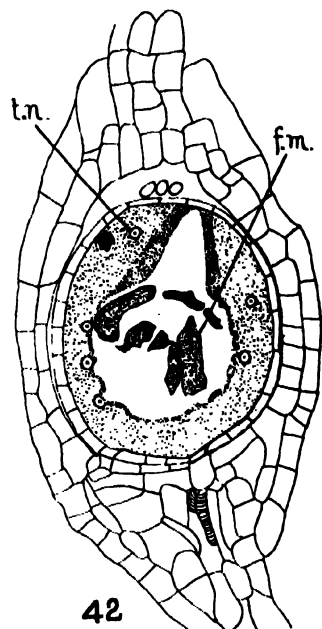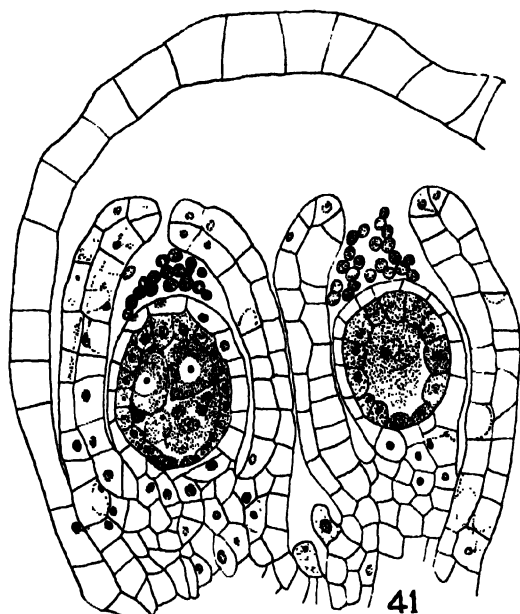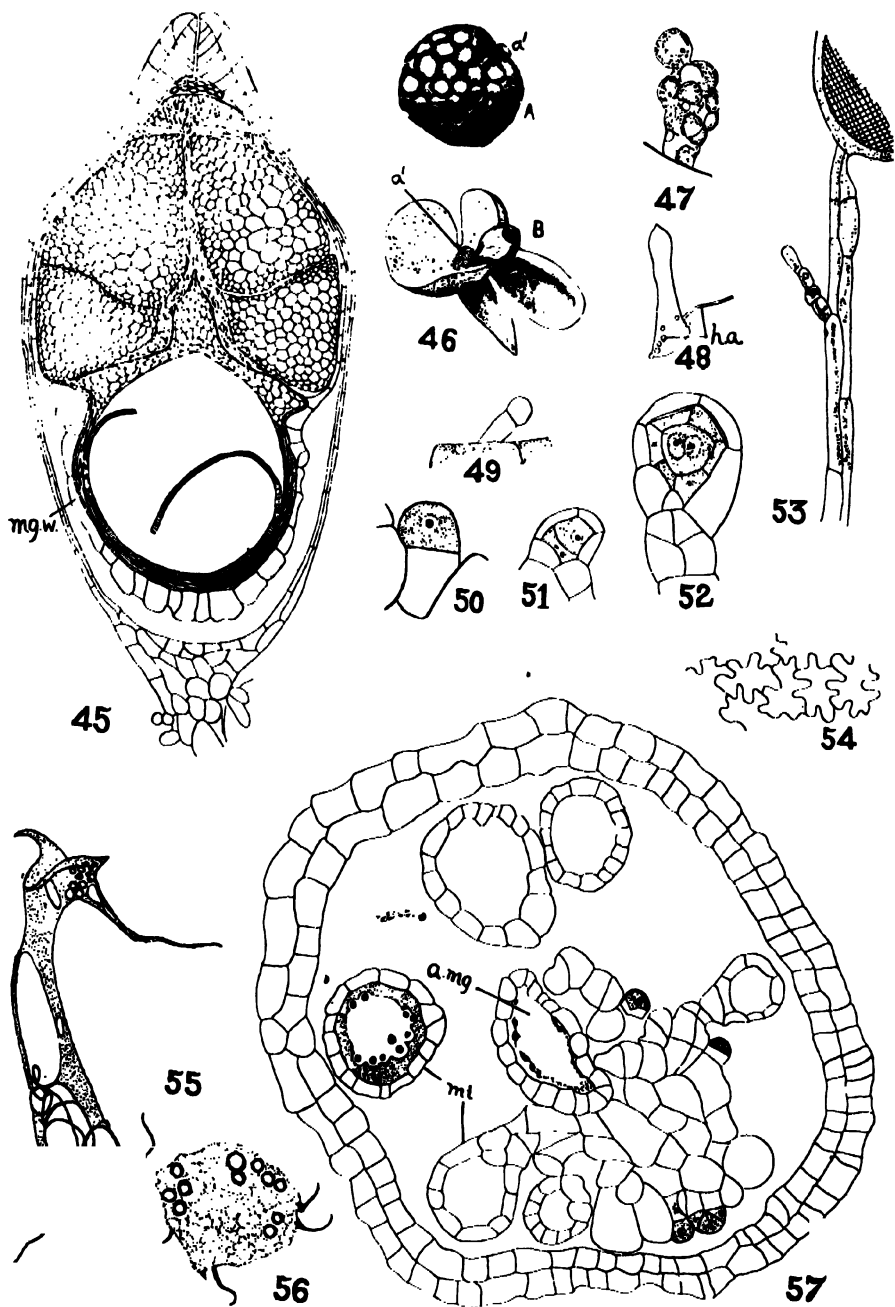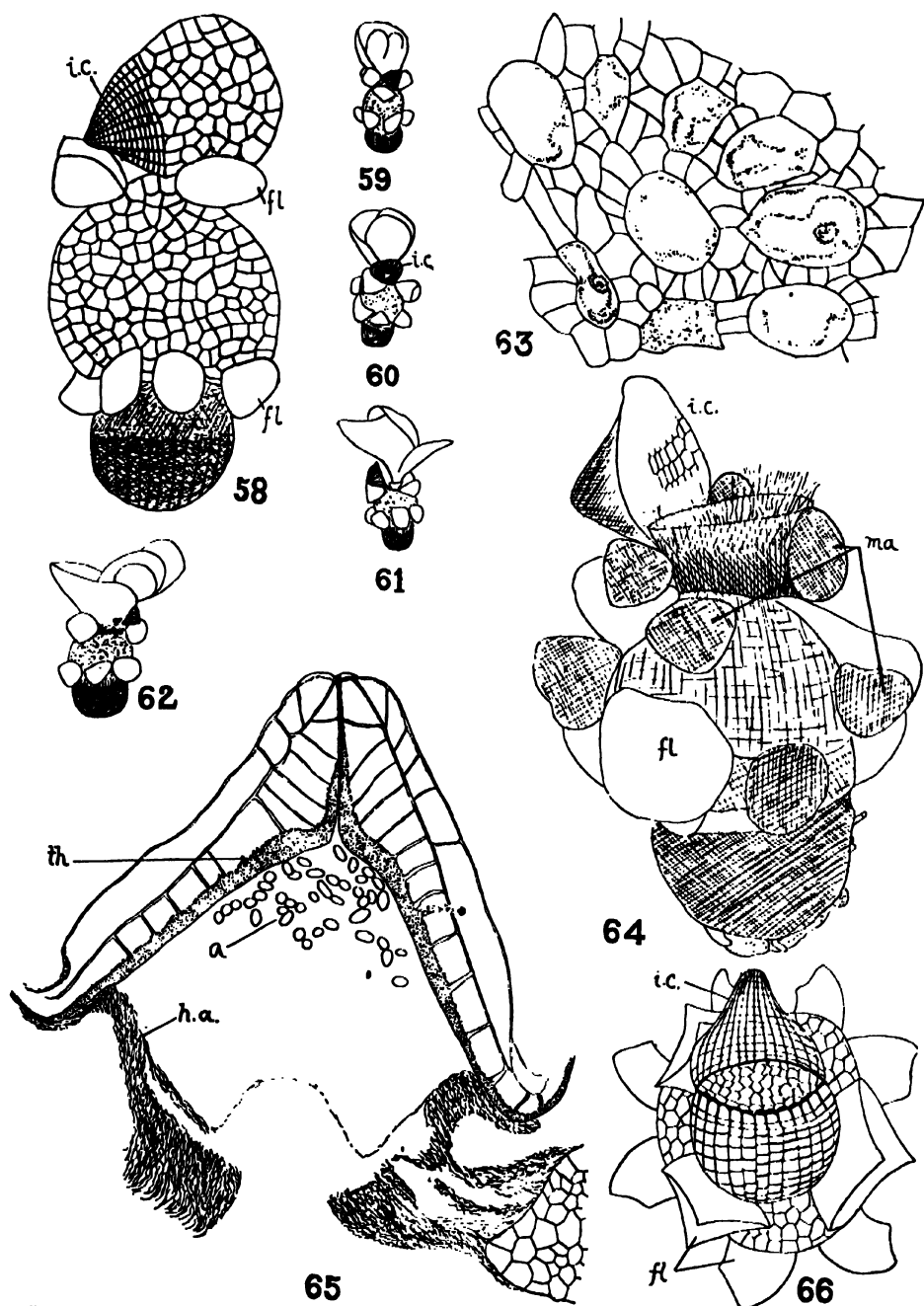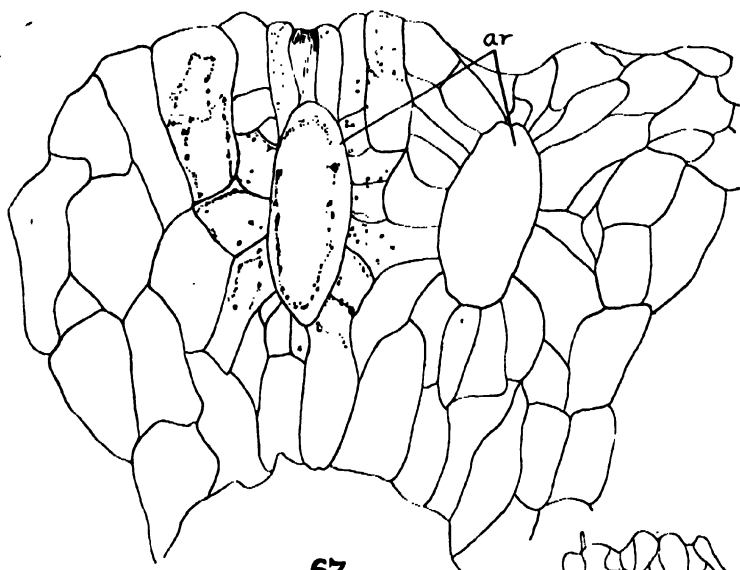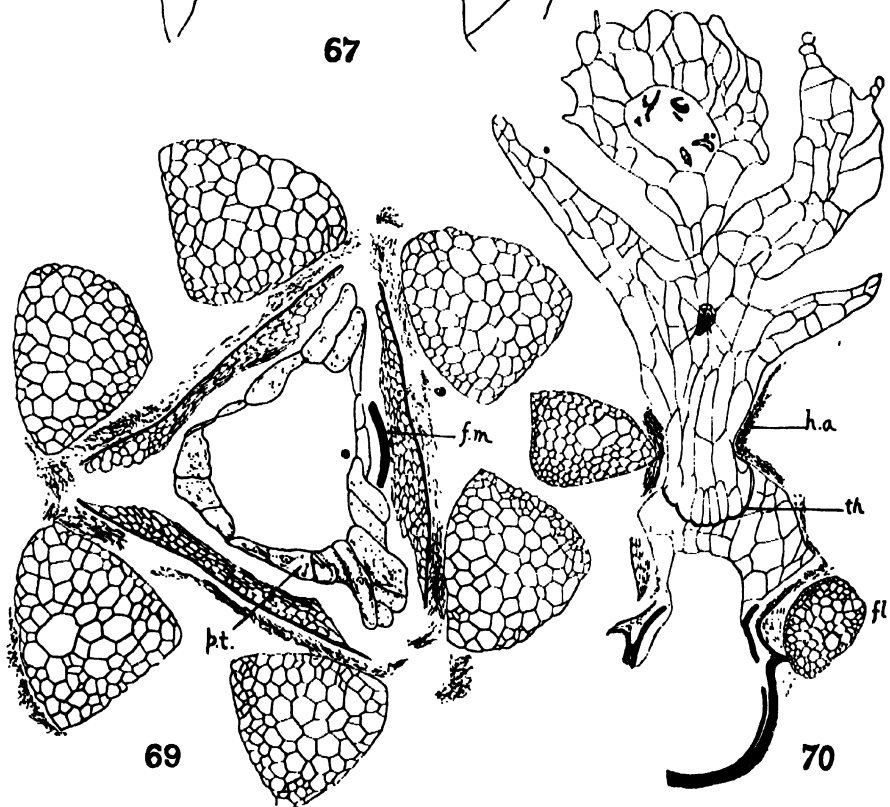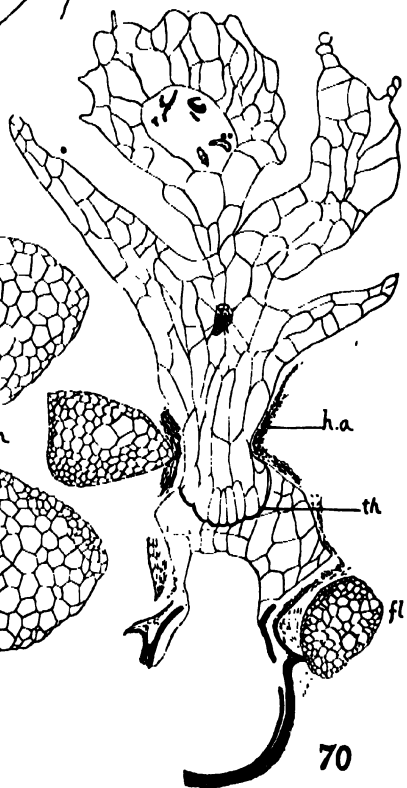
INVESTIGATIONS ON THE RÔLE OF SILICON IN PLANT NUTRITION.

Part II. Adsorption of Silica in Soluble Forms by Colloidal Oxides of Iron and Aluminium.

By A. Sreenivasan, M.A.,

(From the Department of Biochemistry, Indian Institute of Science, Bangalore.)

In a previous communication (Sreenivasan, 1935) it has been shown that when a soluble silicate is added to the soil, part of it, depending upon the concentration in solution, the nature of the soil and, to some extent, its moisture content, is rendered insoluble almost immediately after addition and that the seat of interaction between the soil and silicate is in the colloidal fraction and is probably almost entirely due to the mineral colloids of the soil. Since when Van Bemmelen (1879, 1888, 1900, 1904) pointed out the colloidal properties of the soil, several investigators have studied adsorption phenomena in clays where these latter have been treated as a whole, but in most of the work thus far done, the systems have been so complex that it has not been possible to say whether one had physical adsorption or chemical combination or both. It was considered therefore that a better insight into the nature of interaction between the soil and soluble silicates could be obtained by working with pure colloids. The natural inorganic colloids of the soil are composed mainly of colloidal silica, iron oxide and alumina. In fact they represent adsorption compounds of indefinite composition formed by the mutual precipitation of colloidal hydroxides of opposite charges. A few workers have studied the adsorption of various salts by pure colloids like iron oxide and alumina. Thus Gordon and his co-workers (1922, 1923), Miller (1928), Ghosh and Bhattacharya (1930) and others have thrown useful light on the rôle of soil colloids in plant nutrition and as to how some salts in certain forms become available for plant food.

In the present investigation, adsorption of silica from solutions of sodium silicate and from silica sol by pure hydrogels of iron oxide and alumina has been studied. The adsorption by these colloids of silica under different hydrogen-ion concentrations has also been followed as it was hoped that

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such work might explain how acidity or alkalinity of soil affects silicate adsorption and its usefulness, direct or indirect, as plant food.

Experimental.

Hydrogels of iron oxide and alumina were prepared by addition of ammonium chloride and ammonia to solutions of ferric chloride and aluminium sulphate respectively. The precipitates were allowed to settle and washed by repeated decantation with distilled water until the supernatants were free from ammonia and chloride or sulphate as the case may be. As much of the water as possible was now syphoned off and the suspensions stocked as such.

Sodium silicate solution was made by diluting Kahlbaum's 25 per cent. sodium silicate to ten times its volume and stocking it as such after filtration. The silica in a known volume of this solution was determined in the manner previously described (Sreenivasan, *loc. cit.*).

Silica sol was obtained in pure condition by continued dialysis, for over 10 days, of a mixture obtained by slowly adding sodium silicate solution to

TABLE I.

Silicate added (as mg. of silica in 200 c.c. of solution)	Initial P_H of silicate solution	Silicate in solution after adsorption (as mg. of silica)	Silica retained by gel (mg.)	Per cent. retention of silica	Retention of silica per g. of dry gel (mg.)	P_H of silicate solution after adsorption
Ferric hydroxide gel = 376·0 mg. Fe_2O_3. P_H = 7·6.						
75·1	8·6	10·0	65·1	86·7	173·2	8·4
125·1	9·0	42·0	83·1	66·4	221·0	8·6
250·2	9·2	130·1	120·1	48·1	319·4	8·8
375·3	9·5	224·2	151·1	40·3	401·9	9·2
500·4	9·7	327·9	172·5	34·5	458·6	9·6
Alumina gel = 157·0 mg. Al_2O_3. P_H = 8·0.						
75·1	8·6	23·9	51·2	68·1	326·1	8·6
125·1	9·0	55·8	69·3	55·3	441·2	8·8
250·2	9·2	160·0	90·2	36·1	574·5	9·2
375·3	9·5	280·1	95·2	25·4	606·3	9·6
500·4	9·7	367·9	132·5	26·5	843·9	9·8

dilute hydrochloric acid until the latter was just in excess of the former. Dialysis was done in a parchment membrane and until free from chloride.

Effect of addition of different quantities of sodium silicate to iron and alumina gels.—10 c.c. of each of the gels (containing respectively 376 and 157 mg. of iron oxide and alumina) were taken in stoppered bottles and treated with 200 c.c. of a solution containing different known quantities of silicate. The mixture was in each case shaken repeatedly and let stand overnight so that equilibrium might be attained. They were then filtered and the silica in aliquots estimated. The H-ion concentration of the silicate solution before and after adsorption was also determined colorimetrically using a Hellige comparator outfit. The total silica in solution was calculated, the water present in the gels being also taken into account so that the retention of silica by the gels was expressed on their dry weight. The results are given in Table I.

In another set of experiments, identical quantities of the silicate as in the above, but in 100 c.c. of solution were added to the same weights of the gels so that although the amount of silicate was the same as before, its concentration was in each case double that previously used. The results are given in Table II.

TABLE II.

Silicate added (as mg. of silica in 100 c.c. of solution)	Iron hydroxide = 376·0 mg. Fe₂O₃.				Alumina = 157·0 mg. Al₂O₃.			
	Silicate in solution after adsorption (as mg. of SiO₂)	Silica retained by gel (mg.)	Retention of silica per cent.	Silica retained per g. of dry gel (mg.)	Silicate in solution after adsorption (as mg. of SiO₂)	Silica retained by gel (mg.)	Retention of silica per cent.	Silica retained per g. of dry gel (mg.)
125·1	39·2	85·9	68·7	228·4	43·6	81·5	65·2	519·2
200·2	70·0	130·2	65·0	346·1	76·7	123·5	61·7	786·5
250·2	114·1	136·1	54·4	361·9	132·2	118·0	47·2	751·6
375·3	220·1	155·3	41·4	413·0	271·7	103·6	27·5	659·8
500·4	306·4	194·0	38·9	515·9	342·4	158·0	31·6	1007·0

It would be seen from Tables I and II that adsorption similar to that observed in the case of soil (Sreenivasan, *loc. cit.*) takes place also in the case of the gels of iron oxide and alumina. While as with the soil, the percentage adsorption decreases with increasing amounts of added silicate, a comparison

between Tables I and II will show that adsorption is greater for the same amount of added silicate when it is present in half the volume of the solution. A comparison of the figures for the retention of silica per gram of dry gel would show that alumina retains nearly twice as much of silica as iron hydroxide gel.
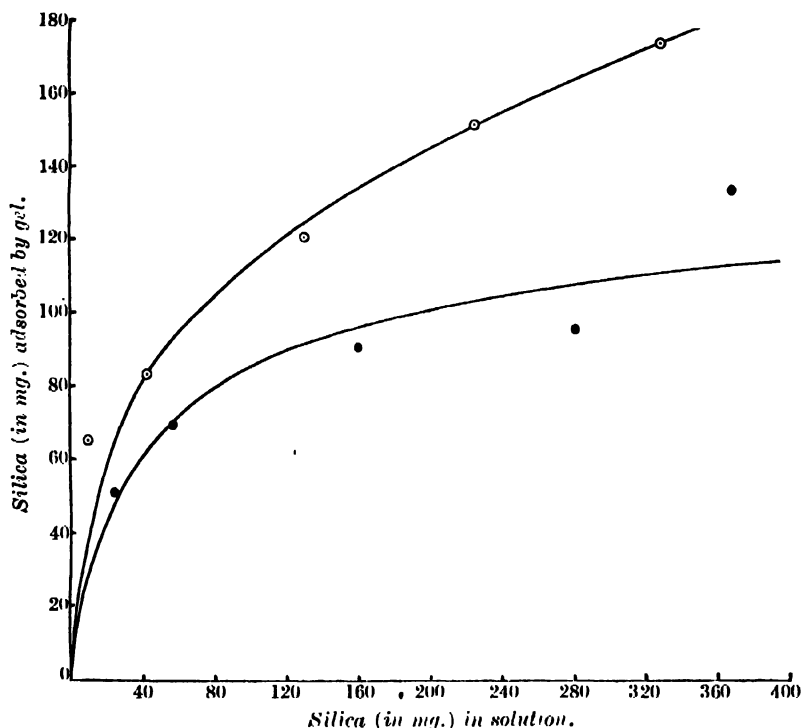
Fig. 1. Adsorption of silicate by gels of iron oxide and alumina.
o—o Ferric hydroxide gel.
●—● Aluminium hydroxide gel.

In Fig. 1, the amount of silica retained by the gels of alumina and iron hydroxide (Table I) have been plotted against the corresponding quantities present in solution. It is seen that the process is an ordinary adsorption obeying more or less the usual logarithmic proportionality law expressed by Freundlich (cf. Sreenivasan, loc. cit.).

In the above experiments the quantities of silicate added were probably far in excess of the actual weights of the adsorbing compounds—viz., iron oxide and alumina and hence the results may not be strictly comparable to the previous studies on soil and silicate solutions. Hence in the following experiments the proportion of hydrogels to silicate was increased. The amount of silica in solution was estimated as before (Table III).

TABLE III.

Silicate added (as mg. of silica in 150 c.c. of solution)	Iron hydroxide = 1432 mg. Fe$_2$O$_3$				Alumina = 1056 mg. Al$_2$O$_3$			
	Silicate in solution (as mg. of SiO$_2$)	Silica retained (mg.)	Percentage of silica retained	Silica retained per g. of dry gel (mg.)	Silicate in solution (as mg. of silica)	Silica retained (mg.)	Percentage of silica retained	Silica retained per g. of dry gel (mg.)
122·3	14·2	108·1	88·4	75·5	12·6	109·7	89·7	103·9
244·6	42·4	202·2	82·7	158·4	36·1	208·5	85·3	197·6
366·9	69·8	297·1	81·0	207·5	71·9	295·0	80·4	279·4
489·2	96·7	392·5	80·2	274·1	88·6	400·6	81·9	379·3

As in previous experiments quite a large part of the added silicate is retained by the colloidal hydroxides. It would be seen, however, that with increasing concentration of silicate the fall in percentage retention is not so rapid as in previous experiments.

Adsorption of silica from silica sol by hydrous iron oxide and alumina.— In the following experiments known quantities of silica sol were used instead

TABLE IV.

Silica sol added (as mg. of silica)	Initial P$_H$ of silica sol	Silica in solution after adsorption (mg.)	Silica retained by gel (mg.)	Per cent. retention of silica	Silica adsorbed per g. of dry gel (mg.)	P$_H$ of silica sol after adsorption
Iron hydroxide gel = 286·4 mg. Fe$_2$O$_3$						
44·6	4·8	13·9	30·7	68·8	107·2	7·0
89·1	5·8	81·5	57·6	64·7	201·1	6·8
133·7	6·2	52·0	81·7	61·1	285·2	6·4
178·2	6·2	99·9	78·3	44·1	273·4	6·4
Alumina gel = 211·2 mg. Al$_2$O$_3$						
44·6	4·8	8·0	36·6	82·1	173·3	7·4
89·1	5·8	28·0	61·1	68·6	289·3	7·0
133·7	6·2	47·2	86·5	64·7	409·6	7·0
178·2	6·2	66·6	111·6	62·6	528·3	6·8

of sodium silicate. The procedure was the same as before. The results are given in Table IV.

The P_H of the silica sol itself was $4 \cdot 0$ while that of the $1 : 10$ diluted sol was $5 \cdot 8$.
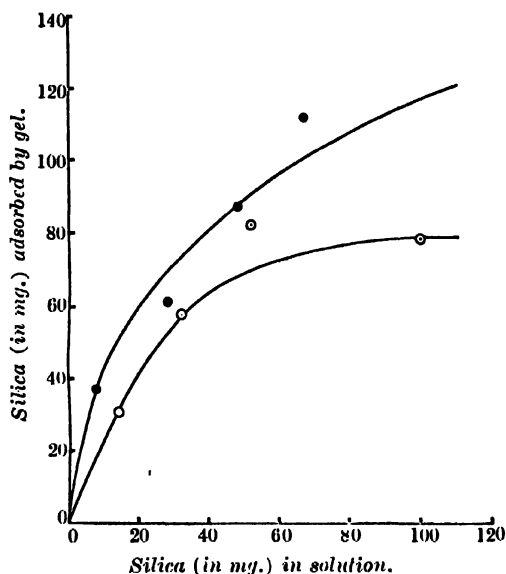
FIG. 2.   Adsorption of silica sol by hydrogels of iron oxide and alumina.
o—o Ferric hydroxide gel.
•—• Alumina gel.

The quantities of silica adsorbed by the gels have been plotted against the corresponding amounts in solution (Fig. 2).   The results thus show that even when silica sol is used instead of sodium silicate, adsorption occurs to a considerable extent.   In this respect silicate adsorption is different from phosphate adsorption because the latter is adsorption of an anion, whereas the present phenomenon would appear to be that of a negative colloidal complex by a positive colloid.

Influence of hydrogen-ion concentration on the extent of retention of silica by gels of iron oxide and alumina.—Since it is known that hydrions and hydroxylions are the most strongly adsorbed ions, it would follow that the adsorption of silica by the colloidal oxides of aluminium and iron would be modified in the presence of one or other of these ions.   It would be of interest therefore to determine the extent of adsorption of silica under varying hydrogen-ion concentrations.

Known amounts of the hydrogels of iron oxide and alumina were treated with 200 c.c. of a silicate solution containing a definite quantity of silica but adjusted to different hydrogen-ion concentrations by means of decinormal hydrochloric acid or sodium hydroxide as the case may be. The mixture was well shaken and after allowing to let stand overnight, filtered and the silica in filtrate determined. Tables V and VI give the results obtained using different amounts of the two gels and different concentrations of the silicates.

TABLE V.

Initial P_H of silicate solution (200 c.c.)	Iron hydroxide gel = 376·0 mg. Fe_2O_3					Alumina gel = 157·0 mg. Al_2O_3				
	P_H of solution after adsorption	Silica in solution (mg.)	Silica retained (mg.)	Per cent. retention of silica	Silica retained per g. dry gel. (mg.)	P_H of silicate after adsorption	SiO_2 in solution (mg.)	Silica retained (mg.)	Per cent. retention of silica	SiO_2 retained per g. dry gel (mg.)
2·5	2·8	304·8	195·6	39·1	520·1	2·8	347·2	153·2	30·6	975·9
5·0	5·8	311·6	188·8	37·7	502·3	6·0	362·0	138·4	27·7	881·6
7·0	8·4	318·8	181·6	36·3	482·9	8·4	369·2	131·2	26·2	835·6
9·0	9·6	327·2	173·2	34·6	460·6	9·8	366·4	134·0	26·8	853·5
10·6	10·0	337·0	163·4	32·7	434·6	10·2	354·0	146·4	29·3	932·8

Silicate added (as mg. of silica) - 500·4.

TABLE VI.

Initial P_H of silicate solution (100 c.c.)	Iron hydroxide gel - 572·8 mg. Fe_2O_3					Alumina gel = 422·4 mg. Al_2O_3				
	P_H of solution after adsorption	SiO_2 in solution (mg.)	SiO_2 retained (mg.)	Per cent. retention of silica	SiO_2 adsorbed per g. dry gel (mg.)	P_H of solution after adsorption	SiO_2 in solution (mg.)	SiO_2 retained (mg.)	Per cent. retention of silica	SiO_2 adsorbed per g. dry gel (mg.)
4·0	5·2	18·0	104·3	85·3	182·1	5·2	14·0	108·3	88·5	256·4
5·0	6·4	20·4	101·9	83·4	177·8	6·6	19·6	102·7	84·0	243·2
7·0	7·4	22·6	99·7	81·5	174·1	7·8	24·2	98·1	80·2	232·3
9·0	7·8	29·2	93·1	76·2	162·5	8·4	26·4	95·9	78·3	227·1
10·6	8·6	41·0	81·3	66·5	141·9	8·8	24·0	98·3	80·4	232·8

Silicate added (as mg. of SiO_2) — 122·3.

It would be seen from the above that as the P_H of the silicate solution changes from the acid side towards alkalinity, there is a gradual decrease

in the percentage retention of silica by iron gel, while with alumina there is also a decrease as the P_H increases though when the reaction is very alkaline, as at P_H 10·2, retention of silica appears to increase again. It is probable that, although the extent of hydrolysis of sodium silicate will be far less in both acid and alkaline regions the increased retention of silicate observed at low P_H may be due to a part of the iron or alumina gel being brought into solution only to be precipitated again as the corresponding silicate. Since alumina gel is amphoteric and is therefore soluble at very high P_H as well, this will explain the high retention of silica by alumina in alkaline medium. With iron hydroxide, however, at high P_H no iron is soluble and, besides, there will be a suppression of hydrolysis of the sodium silicate. The adsorption of silica is therefore less in this case. It may be noted that other workers (Mattson, 1927, 1931 ; Gordon and co-workers, *loc. cit.*) have made similar observations with regard to the adsorption of phosphate ion by soil colloids and found that adsorption decreases with increased P_H values and *vice versa.*

Influence of Hydrogen-ion concentration on the adsorption of silica from silica sol by gels of iron oxide and alumina.—The use of solutions of sodium silicate of different hydrogen-ion concentrations naturally brings about variations in the extent of hydrolysis of the silicate solution. Hence any change in the retention of silica from sodium silicate solutions of varying P_H may be due to the varying degrees of hydrolysis of the sodium silicate or to the change in reaction itself. In the following experiments pure silica sol adjusted to different H-ion concentrations by addition of decinormal acid or alkali was used so that the effects due to hydrolysis were eliminated.

TABLE VII.

Initial P_H of silica sol	Iron oxide gel = 286·4 mg. Fe$_2$O$_3$					Alumina gel = 211·2 mg. Al$_2$O$_3$				
	P_H of solution after adsorption	SiO$_2$ in solution (mg.)	SiO$_2$ retained (mg.)	Per cent. retention of silica	SiO$_2$ retained per g. dry gel (mg.)	P_H of solution after adsorption	SiO$_2$ in solution (mg.)	SiO$_2$ retained (mg.)	Per cent. retention of silica	SiO$_2$ retained per g. dry gel (mg.)
2·0	3·0	57·4	120·8	67·8	345·9	2·6	37·5	140·7	79·0	297·5
4·0	4·8	80·2	98·0	55·0	280·6	4·0	49·6	128·6	72·2	271·6
5·0	6·0	109·0	69·2	38·8	198·2	6·0	71·1	107·1	60·1	226·2
10·6	9·6	115·0	63·2	35·5	181·0	9·0	65·3	112·9	63·4	238·4

Silica sol added (as mg. of silica) = 178·2.

Known amounts of the hydrogels of iron oxide and alumina were treated in well-stoppered bottles with the same volume (100 c.c.) of a solution of silica sol adjusted as before to different initial P_H values. The mixture was well shaken, let stand overnight and then filtered. The P_H of filtrate was determined and the silica in aliquots estimated. The amount of silica in solution was then calculated, all the water of hydration of the gel being considered as water of dilution in order that minimum adsorption might be shown. The results are given in Table VII.

Similar change in the amounts of silica retained with increasing P_H is observed as in the experiments with sodium silicate. Thus, in the case of iron hydroxide gel there is a regular decrease in adsorption as the P_H increases, but with alumina there is a decrease followed by a slight increase at high P_H. The decrease in adsorption with increased P_H may be due to the presence of hydroxyl ions which might be preferentially adsorbed by the gels. Alumina gel, not being so electro-positive in alkaline medium as iron gel, would have less affinity for hydroxyl ions and consequently more of silica is retained.

Successive washings of the gel-silica complex.—Known amounts of the gels of iron oxide and alumina were treated in stoppered bottles with definite volumes of sodium silicate, shaken well and allowed to remain overnight. The mixture was then filtered and the residue on the filter paper washed with successive 100 c.c. portions of hot water and the silica in filtrates determined separately. The results are given in Table VIII.

TABLE VIII.

	Iron gel (286·4 mg. Fe_2O_3)	Alumina gel (211·2 mg. Al_2O_3)
Silica added as silicate ..	249·0 mg.	
Silica recovered from solution (mg.)	112·6	134·4
	Silica in 100 c.c. of wash water from gel-silica complex (mg.)	
First 100 c.c.	31	46
Second 100 c.c.	12	10
Third 100 c.c.	4	6
Fourth 100 c.c.	Nil	Nil

The first washings give an appreciable quantity of the silica, probably due to the latter having been mechanically held by the gel and being washed away rather than due to any solvent action on the complex. Subsequent washings give practically no silica in solution, thereby suggesting that a fairly stable adsorption complex is formed.

Discussion.

It has been shown in the previous investigation (Sreenivasan, 1935) that the interaction between soil and sodium silicate solutions is mainly accounted for through adsorption by the mineral soil colloids. The present enquiry with single colloidal materials like hydrous alumina and iron oxide has confirmed that it is through the phenomenon of adsorption that silica is retained by the colloids. This would also show the similarity between the colloidal properties of the soil and those of gels of alumina, iron oxide and silica, first pointed out by Van Bemmelen (*loc. cit.*) and later on emphasised by a number of other workers (Whitney, 1921; Gordon, 1922; Anderson and Mattson, 1925; and others). The colloidal soil material is chiefly made up of silica, alumina, iron oxide, organic matter (humus) and combined water. These divide themselves electro-kinetically into positive and negative colloids. Silica and humus are strictly electro-negative (though silica gel is electro-positive at very low P_H, *i.e.*, in the region of P_H 1·2). They adsorb and combine with bases. The sesquioxides are electrical ampholytes, being electro-positive in acid and electro-negative in alkaline solutions (Mattson, 1930). Thus, the study of the influence of hydrogen-ion concentration has shown that adsorption decreases with increase in P_H. This is to be expected because, due to the presence of excess of hydroxylions as the acidity decreases, the absorptive properties of the hydrogels will be weakened by saturation with hydroxyl ions. In the case of alumina gel, however, silicate retention is greatest both at low and at high P_H. This is of course due to the especially pronounced amphoteric character of that colloid.

Since at low P_H it can be assumed that the hydrolysis of sodium silicate will be negligible and since even then silicate adsorption occurs to a considerable extent, it would follow that silicate adsorption proceeds independently of the extent of hydrolysis of sodium silicate. This would support the earlier view (Sreenivasan, *loc. cit.*) in regard to the composition of sodium silicate, *viz.*, that it is essentially a mixture of alkali and silicic acid sol. It is possible that conductivity determinations of solutions of sodium silicate mixed with varying known amounts of acid and alkali would throw more light on the nature and behaviour of this compound in solution.

The possible beneficial effects of silicate adsorption in releasing from combination certain fertilising ingredients which may not otherwise become available to plant nutrition has been indicated elsewhere (Sreenivasan, 1934). Although the adsorbed silicate is firmly retained by the colloidal hydroxides, being only removed in traces by leaching with water, yet nothing is known as to how far the adsorption complex is stable in the presence of other ions such as the phosphate. Further work along these lines would help to throw light on the mode of action of silica in inducing greater phosphorus intake by plants.

Summary.

(1) When a solution of sodium silicate is added to hydrogels of iron oxide or alumina there is considerable adsorption of silicate similar to that observed in the case of the soil. Retention of silica is greater in the case of alumina than with iron oxide gel and the percentage retention decreases with increasing concentration.

(2) Similar adsorption of silica occurs in the case of ferric, or aluminium hydroxide gel —silica sol systems.

(3) Study of the influence of hydrogen-ion concentration on extent of silica retention shows that as the P_H of the medium increases, there is a decrease in the retention of silica by the gel. With alumina gel at high P_H, however, there is greater adsorption. This is due to the amphoteric nature of alumina.

(4) The adsorbed silicate is firmly retained by the colloidal gel and is not leached out by water.

(5) The possible significance of silicate adsorption in relation to phosphorus resorption in soils is indicated.

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ESTIMATION OF NITROGEN BY FUMELESS DIGESTION. PART I.

By Y. V. Narayanayya, B.A., B.Sc.,

AND

V. Subrahmanyan, D.Sc., F.I.C.

(*From the Department of Biochemistry, Indian Institute of Science, Bangalore.*)

Estimation of nitrogen is perhaps the most important item of analytical procedure in chemical as well as biological research. In the study of plants, animals or micro-organisms, in the evaluation of foods, feeding stuffs or fertilizers or in the examination of water sewage or soil ; determination of nitrogen—in one or more of its several forms— is an essential operation without which no useful conclusions can be drawn. A conservative estimate would, indeed, show that in purely scientific research alone -excluding routine estimations in Government laboratories or in private, consulting or commercial practice for which published records are not generally available—at least a few millions of determinations of nitrogen are being annually carried out by workers in different parts of the world.

Among the various methods for the estimation of nitrogen, the one that is most generally adopted is that originally developed by Kjeldahl (1883) and subsequently modified by several workers (*e.g.*, Arnold, 1887 ; Gunning, 1889). The procedure is comparatively simple and can be easily followed even in routine operations. It is, on the other hand, slow and tedious, especially when comparatively resistant materials like soils, yeasts, or cereal husks have to be digested. It is also attended by the emission of acid fumes which is perhaps its most objectionable feature.

In recent years, a number of attempts have been made to further simplify the original Kjeldahl method or to hasten the process of digestion. Particular mention should be made of the work of Bal (1925), who showed that addition of water increases the efficiency of digestion. This was followed by the researches of Sreenivasan (1932, 1933 and 1934) who explained the mechanism of the retention of nitrogen during 'dry' digestion and showed that pre-treatment with water combined with small quantities of oxidising agents such as peroxides, dichromates, permanganates or perchlorates, not only hastens the rate of digestion, but also improves the estimate of nitrogen. Valuable contributions have also been made by workers who sought to combine the

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wet combustion of carbon with the estimation of nitrogen in the residue (Kruger, 1894 ; Fritsch, 1896 ; Robertson, 1916 ; Anderson and Schutte, 1924 ; Brown, 1927 ; Antipov-Karataiev and Fillipova, 1932 ; Tiurin, 1933 ; Subrahmanyan, Narayanayya and Bhagvat, 1934 ; Robertson and Shewan, 1935 ; Shewan, 1935 ; and Narasimha Acharya, 1935). Their conclusions have been rather discordant, but the recent findings of Narayanayya and Subrahmanyan (1935) would show that, under certain conditions, digestion of nitrogen proceeds to completion in presence of diluted acid and oxidising agents. A simple, fumeless method of estimating nitrogen based on the above principle has been developed and will be described in the present paper.

Experimental.

Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*) have already drawn attention to the fact that the residue left after the wet combustion of carbon gives lower estimates for nitrogen than those obtained by either the official method of 'dry' digestion or the 'wet' procedure described by Sreenivasan and Subrahmanyan (1933). Further experiments with some representative specimens of Indian soils yielded the following results (Table I).

TABLE I.

Description of Soil	Total Nitrogen in parts per million		On the residue after wet combustion of carbon
	As estimated by		
	' Dry '* digestion	' Wet ' digestion	
Godavari delta—Alluvial ..	698	717	639
Bangalore—Sandy loam ..	536	566	506
Nagpur—Heavy black ..	639	699	614
Sindh—*kalar* (alkali) ..	590	604	532
Mandalay—Irrigated rice land	518	536	188
Punjab—Rainfed—Surface..	608	632	578

* Gunning and Hibbard, "Methods of Analysis," *A.O.A.C.*, 1930.

These observations are in general agreement with those of Robertson (*loc. cit.*) and the more recent findings of Shewan (*loc. cit.*).

Effect of continuing the digestion after oxidation of carbon.—Since the ordinary Kjeldahl digestion of soils takes several hours, it appeared probable

that the wet combustion of carbon which occupies less than 30 minutes may not provide sufficient heating which the complete digestion of the different forms of nitrogen may require. With a view to verifying this, some experiments were carried out treating four different specimens of soils (10 g. each) with potassium dichromate (10 g.), water (20 c.c.) and concentrated sulphuric acid (40 c.c.) and digesting them on the sand bath for varying periods of time. The results have been presented in Table II.

TABLE II.

Soil from	Total Nitrogen in parts per million				
	Expected*	After oxidative digestion for			
		30 mins.	60 mins.	90 mins.	120 mins.
Bangalore	566	506	506	500	500
Punjab	632	578	578	572	575
Godavári delta	717	639	612	639	636
Sindh (*kalar*)	604	510	532	536	530

* Sreenivasan and Subrahmanyan, *Indian J. Agric. Sci.*, 1933, **2**, 646.

There was considerable difficulty in continuing the digestion after 30 minutes. There was much separation of sand and other mineral matter which caused violent bumping. The results show that even prolonged heating under such conditions led to no improvement in the estimate of nitrogen.

The low estimates of nitrogen obtained in the previous experiments may have been due to (*a*) retention of nitrogen in some resistant form in either liquid digest or in the insoluble residue, and/or (*b*) conversion of a portion of the combined nitrogen to nitric acid or gases such as nitrogen peroxide (NO_2), nitric oxide (NO), or even elementary nitrogen. Retention of nitrogen in the digest is known in cases when mercury is used to catalyse the digestion. Sreenivasan and Subrahmanyan (*loc. cit.*) have shown that silica and even increasing quantities of ferric oxide and alumina may retain some nitrogen in the digest. Moreover, the existence of complex ammines, such as those of chromium are known, though their stability in presence of hot, concentrated sulphuric acid and, subsequently, during distillation with concentrated alkali is uncertain. The distillate collected even after prolonged boiling of mixtures

of soil (a specimen from Bangalore), acid and oxidising agent, failed to reveal the presence of either nitric oxide or nitrogen peroxide. Nitric acid was present in no more than traces and this may have been partly derived from the original soil itself. Vigorous heating of mixtures of ammonium sulphate and potassium dichromate or ammonium dichromate alone with sulphuric acid and water failed to show any appreciable loss of nitrogen. The last observation is apparently contradictory to the findings of Shewan, but its significance will be discussed in a subsequent communication. The digestion itself could not be incomplete as, in all the cases, the organic matter was fully oxidised in the course of the first few minutes (Bhagvat, Narayanayya and Subrahmanyan, *loc. cit.*).

Effect of prolonged heating of the insoluble residue left after digestion.— Samples (10 g.) of four soils were weighed into a number of Kjeldahl flasks and treated with dichromate (10 g.), water (20 c.c.) and sulphuric acid (40 c.c.). The mixtures were boiled for half an hour to ensure complete oxidation of all organic matter. The digests were then divided into two batches in one of which, after the necessary dilution, the nitrogen contents of the dissolved portion and the insoluble residue were determined separately by distilling with excess of alkali ; in the other, the insoluble residue was separated and then treated with water (20 c.c.) and further quantity of sulphuric acid (20 c.c.) and wet digested according to Sreenivasan and Subrahmanyan (*loc. cit.*). The results have been presented in Table III.

TABLE III.

Soil from	Total Nitrogen in parts per million			
	Expected	Found by oxidative digestion		In the residue after further digestion
		In the supernatant	In the residue	
Bangalore ..	566	227	279	279
Sindh ..	722	451	211	211
Mandalay ..	536	421	67	70
Punjab ..	632	482	96	96

The foregoing observations show that the nitrogen retained in the precipitate, if any, is not released on prolonged digestion. The mode of retention would also appear to be of a different type from that recorded by previous

workers (Bal, *loc. cit.* ; Sreenivasan and Subrahmanyan, *loc. cit.*) for ' dry ' digested soils.

Effect of chromium sulphate on the efficiency of digestion.— Since the dichromate ultimately forms potassium and chromium sulphates in the acid medium and since potassium sulphate does not interfere with the progress of digestion, it was considered probable that the formation of the chromium salt may, in some way, be related to the lowering of the estimates. Some experiments were accordingly carried out adding chromium sulphate (10 or 20 g.) in place of the potassium salt to three different types of soils and conducting the Kjeldahl digestion in the usual way. The results have been given in Table IV.

TABLE IV.

Soil from	Total Nitrogen in parts per million		
	With K_2SO_4 (control)	With $Cr_2(SO_4)_3$	
		10 g.	20 g.
Bangalore ..	566	456 ; 500	430 ; 510
Sindh ..	666	529 ; 560	508 ; 572
Godavari Delta ..	717	580 ; 628	600 ; 636

The digestion was rendered very difficult owing to the rapid separation of chromium sesquioxide. There was a tendency to cake at the bottom. Bumping was also rather violent. Owing largely to this, the results of the duplicate determinations were quite discordant. In no case, however, was the value so high as that obtained in the corresponding control.

Effect of pre-treatment of soil.—It appeared probable that, as in some of the previous studies, the low values obtained with dichromate may be due to inadequate penetration of the soil by the reagents. Some experiments were accordingly carried out treating the soil-dichromate mixture (10 g. each) with 30 c.c. of water and allowing the suspensions to stand for varying periods of time (4 to 24 hrs.), before adding sulphuric acid. The results which have been given in Table V show that the low values are not due to insufficient penetration, at any rate, of the type described by the previous workers. This conclusion is further supported by the parallel studies of Rajagopal (private communication) on yeast.

TABLE V.

Soil from	Total Nitrogen in parts per million				
	Expected	Oxidative digestion after pre-treatment with water for			
		4 hrs.	8 hrs.	12 hrs.	24 hrs.
Bangalore ..	566	500	510	506	509
Mandalay ..	536	480	490	485	493
Sindh ..	666	550	560	585	565

Effect of adding chemical precipitants.—It is well known that the nitrogen retained by mercury salts present in the digest can be released by a suitable precipitant such as potassium sulphide. It was considered probable, therefore, that treatment with certain chemicals either during digestion or just prior to distillation with alkali might lead to the release of any nitrogen that may be held in combination. Some trials were accordingly carried out, the details relating to which, as also the results, have been given in Table VI.

TABLE VI.

Treatment	Total Nitrogen in parts per million	
	Bangalore soil	Sindh soil (2nd sample)
'Wet' (Kjeldahl) digestion (control) ..	566	666
Oxidative digestion ..	506	570
Potassium Sulphide (100 g.) added just prior to distillation	500	555
Sodium (ordinary) Phosphate (100 g.) added just prior to distillation ..	496	560
Oxidative digestion with HgSO$_4$ (15 g.)	398	462
Do. BaSO$_4$ (,,)	500	563
Do. CuSO$_4$ 5H$_2$O (,,)	510	566
Do. PbSO$_4$ (,,)	500	563

It may be noted that none of the treatments led to any improvement in the estimate of nitrogen. There was, in fact, marked depression in the case of specimens treated with mercuric sulphate.

Effect of treatment with reducing agents.— Samples (10 g.) of three different soils were wet combusted according to Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*), the digests transferred to distilling flasks and diluted to about 200 c.c. in each case. They were then treated with 10 g. of tin, magnesium or zinc and boiled for 30 minutes. The mixtures were cooled and distilled with excess of alkali in the usual way. Blank determinations were also carried out with the metals alone. The estimates for total nitrogen obtained after making necessary corrections are given in Table VII.

TABLE VII.

Soil from	Total Nitrogen in parts per million				
	Expected	As found by oxidative digestion	Oxidative digestion followed by reduction with		
			Tin	Magnesium	Zinc
Bangalore ..	566	506	520	510	566
Punjab ..	632	578	589	606	635
Mandalay ..	536	488	502	520	540

Tin was comparatively slow in action and was not fully acted on even after 30 minutes of boiling. Magnesium, on the other hand, was very violent in action. The powdered metal tended to float on top and reacted at the surface of the acid with considerable evolution of heat. The effect of that metal as a reducing agent was not, therefore, very pronounced. Zinc was much less violent in its action, but reacted with the acid at a steady rate, and at the end of about 15 minutes, its action was generally complete. The results obtained with that reducing agent were highly satisfactory and corresponded closely to those of the control.

When the above procedure was extended to different types of soils, it was found that correct estimates could not always be obtained. The results varied considerably with the rate of heating. It was found, in consequence, that the volumes of the digests changed with each experiment. As the efficiency of digestion depends, to a large extent, on the constancy of the proportion of acid to water, some experiments were carried out adding water

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from time to time to the digesting mixtures and thus maintaining the total volume more or less constant. As this procedure led to greatly improved results, the digestions were next carried out fitting the Kjeldahl flasks with air- or water-cooled condensers. This modification led to accurate estimates being obtained in all the cases. The following results will illustrate the advantages of combining refluxing during digestion with reduction of digest prior to distillation (Table VIII).

TABLE VIII.

Description of soil	Total Nitrogen in parts per million			
	Expected	Found after reduction with zinc alone (digest not refluxed)	Found after refluxing unaccompanied by reduction	Refluxing combined with reduction with zinc
Sindh—(Kalar) soil ..	572	512	445	572
Nagpur—Black cotton soil	626	572	626	626
Bangalore—Sandy loam ..	566	566	566	566

It may be seen from the above that one soil requires only reduction, while another requires only refluxing. The third requires both the treatments. As the probable behaviour of a soil cannot be easily anticipated, it would be desirable to combine both the treatments in all the cases.

The improvement obtained from refluxing would suggest that, at any rate, in a few cases, the digest contains volatile compounds of nitrogen. Although it was found in some of the earlier experiments that no more than traces of nitric acid were formed from some of the samples that were tried, it is yet probable that others may form that acid in sufficient quantity to affect the estimates of total nitrogen. Moreover, certain types of soils may be naturally rich in nitrates. The nitric acid released on boiling with sulphuric acid may volatilise if the digestion is carried out without proper condensation of the ensuing vapours.

The foregoing observations are in agreement with the recent findings of Narasimha Acharya (loc. cit.) and Harihara Iyer and Rajagopalan (private communication). The latter authors have indeed observed that even added nitrates in small quantities can be retained in the digest and included in the estimate of the total nitrogen if the mixture is refluxed during boiling and subsequently reduced in either acid or alkaline medium.

The mechanism of the action of Zinc.—The improved estimate resulting from treatment with zinc may be due to (*a*) reduction of nitrogenous compounds formed in the course of oxidation, and/or (*b*) dissolution of a part of the precipitate which may otherwise retain some of the nitrogen. The latter is, however, hardly probable since, as already observed, oxidative digestion of yeast yields no precipitate, though the digest has to be treated with zinc before correct estimates of nitrogen can be obtained. The related process seems therefore to be largely one of reduction. As already explained, this conclusion is supported by the presence of minute quantities of nitric acid in the digest. The available evidence is not sufficient, however, to state whether any complex ammine is formed during digestion. The observations with other reducing agents may be summarised as follows. Brass (in the form of powder) acts in acid medium, but is not so effective as zinc alone. Treatment with reduced iron in acid medium yields correct estimates of nitrogen, but the quantity of precipitate formed on addition of alkali is quite considerable so that the subsequent distillation of ammonia is rendered difficult. Aluminium does not act in acid medium, but is very effective in presence of alkali. The latter reaction is, however, very violent, so that there is always the danger of alkali spray being mechanically carried over during the distillation. Similar remarks would also apply to Devarda's alloy, though its action is less violent than that of aluminium. Treatment with milder reducing agents like stannous chloride or oxalic acid does not lead to any marked improvement over the control. It may be concluded from the above that correct estimates of nitrogen can be obtained only by treatment with substances forming nascent hydrogen in either acid or alkaline media.

Influence of proportion of acid and water on the accuracy of the estimate of nitrogen.—It was observed that the digestion was comparatively slow when the proportion of acid to water was as 1 : 1 by volume. Even at the end of one hour, the digestion was often incomplete. Increasing the proportion of acid to correspond to the ratio 2 : 1 led to very much more rapid digestion, the entire process being generally complete in about 15 minutes. In no case was it necessary to extend the digestion beyond 30 minutes. Further increase in the proportion of acid to water (4 : 1, 6 : 1 or 8 : 1) did not lead to any corresponding increase in the rate of digestion. The proportion 2 : 1 was therefore adopted in the subsequent studies. In this connection, it may be of interest to mention that Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*) found the same proportion to be best suited for the wet combustion of carbon.

Estimation of Nitrogen in soils containing chlorides.—Anderson and Schutte (*loc. cit.*) have drawn attention to the fact that the estimate of nitrogen

obtained on the residue after wet combustion of carbon is greatly lowered by the presence of chlorides. According to those authors, chlorides react with the ammonia in the digest producing ammonium chloride, which, on interaction with the unused potassium dichromate, evolves gaseous nitrogen through intermediary formation of ammonium dichromate. Our observations have confirmed their findings in regard to reduced estimates being obtained. Indeed, in some of the earlier experiments when concentrated hydrochloric acid or solid sodium chloride was added with a view to reducing the excess of chromic acid in the digest, practically all the nitrogen was lost as gas. On the other hand, the explanation offered by Anderson and Schutte is untenable, because, even ammonia in solution yields elemental nitrogen on treatment with chlorine. Hypochlorous acid which is formed by interaction with water reacts readily with ammonia or amides so that, under such conditions, correct estimates of nitrogen cannot be obtained unless the production of chlorine through interaction between the chloride and chromic acid is avoided.

The previous observations of Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*) having shown that addition of mercury salts (particularly the oxide and the sulphate) effectively prevents the formation of chlorine under such conditions, some experiments were next carried out with two soils to which known quantities of chloride had been added. The related particulars, as also the results, have been given in Table IX.

TABLE IX.

Soil from	Treatment	Total Nitrogen in parts per million when the digest contains			
		Chloride (0·01 per cent.) as NaCl	Chloride (0·02 per cent.) as NaCl	Chloride (0·05 per cent.) as NaCl	Chloride (0·1 per cent.) as NaCl
Bangalore (Tot. N., 566 p.p.m.)	Without HgSO$_4$	200	60	16	10
	With HgSO$_4$ (2 g.)	420	407	380	400
	With HgSO$_4$+Zinc (2g.)	566	560	566	569
Godavari Delta (Tot. N., 717 p.p.m.)	Without HgSO$_4$	160	100	18	6
	With HgSO$_4$ (2 g.)	520	500	495	510
	With HgSO$_4$+Zinc	717	720	717	717

It may be observed that with increasing quantities of chloride, there was correspondingly greater loss of nitrogen until, with $0 \cdot 1$ g., there was practically none left in the digest. Addition of mercuric sulphate was effective in preventing this loss, though, owing to retention of nitrogen, correct estimates could not be obtained, unless the digests were treated with zinc prior to distillation. There was obviously no need to add any chemical precipitant (such as potassium sulphide) to release nitrogen from combination with mercury. This observation is in agreement with the findings of Böttcher (1892) and, more recently, of Harihara Iyer and Rajagopalan (private communication). Even alkali soils rarely contain more than 2 to 3 per cent. of chlorides. Some of the later observations have shown that by addition of a larger quantity of mercuric sulphate (5 g.), loss of nitrogen from specimens containing upto 5 per cent. of chlorides can be prevented. In all the cases, the digest has to be boiled in zinc in the manner previously described.

Procedure of the estimation of nitrogen in soils.—Based on the results of the foregoing study, a simple, fumeless method of estimating nitrogen has been developed. The advantages of such a procedure over the usual Kjeldahl method may be enumerated as follows.—The time of digestion is reduced to 30 minutes whereas according to the Kjeldahl method it may often take several hours. Emission of fumes is completely avoided and the digestion can be easily conducted at any working bench in the laboratory. Unlike the Kjeldahl digest which often bumps or otherwise requires frequent attention, the oxidative digestion proceeds smoothly and requires no attention. Addition of mercuric oxide or sulphate may not always be necessary but its inclusion in routine practice is recommended so as to avoid loss of nitrogen, if any of the samples should, unexpectedly, contain chlorides. Reduction with zinc is no doubt an extra operation, but that too requires practically no attention. The reduction proceeds rapidly especially when the zinc is finely powdered and is often complete in under 10 minutes. The distillation proceeds smoothly and takes no more than the usual time. It may thus be reckoned that the entire process of determination—from the weighing of the soil to the back-titration of the unused acid—can be completed in about two hours.

With the exception of the soil, none of the chemicals used in the estimation would require accurate weighing. A convenient procedure would be to weigh out the latter beforehand into a number of packets which may be taken out whenever needed.

Application of the method to the estimation of nitrogen in some representative Indian soils.—Following the new procedure, the total nitrogen contents

of some representative specimens of Indian soils were determined. The results, as compared with those obtained by 'wet' digestion, have been given in Table X.

TABLE X.

Locality and description of soil	Total Nitrogen in parts per million as estimated by	
	' Wet ' digestion	The new method
Travancore—Loam, alkaline ..	217	219
Sindh—*Kalar* (saline) ..	572	572
Punjab—Irrigated rice land ..	596	599
Punjab—Rainfed, surface ..	632	629
Nagpur—Heavy, black ..	626	626
Sholapur—Heavy, black ..	243	240
Sholapur—Medium, black ..	415	418
Bangalore—Sandy loam ..	566	566
Ranchi—Upland, surface ..	488	492
South Bihar—Alluvial ..	361	359
Mandalay—Paddy ..	536	536
Jaffna—Sandy ..	325	325

It may be noted that there was close agreement between the two sets of values.

Modification of procedure to include nitrates.—Most soils contain only minute quantities of nitrates so that the estimate of total nitrogen is not appreciably affected even if all the nitrate is lost during digestion. There are, nevertheless, certain types of soils which are usually rich in nitrates and which must be specially treated to obtain accurate estimates of total nitrogen. A critical study of the various methods employed for this purpose was recently made by Sreenivasan (1935), who came to the conclusion that reduction of the nitrate with Devarda's alloy in the cold prior to commencement of digestion is the most effective way of including all the nitrate that may be present in the soil system. With a view to determining whether a

similar procedure can be combined with the oxidative digestion of organic nitrogen, known quantities of nitrates were added to specimens of four soils and, after reduction according to Sreenivasan, the total nitrogen contents were estimated in the manner outlined previously. The results, which have been given in Table XI, show that there is close agreement between the values expected and those actually found.

TABLE XI.

Soil from	Total Nitrogen in parts per million		
	Soil alone	Soil + nitrate (200 p.p.m. of N)	Soil + nitrate (400 p.p.m. of N)
Bangalore ..	566	762	960
Ranchi ..	488	680	878
Godavari Delta ..	717	911	1,105
Jaffna ..	325	· 520	718

Although the procedure adopted in the above experiment yielded accurate results, it was, nevertheless, comparatively slow and tedious. Attempts to hasten the reduction of nitrate by heating proved ineffective, because the resulting ammonia tended to escape through cotton wool soaked in acid which was used as the trap. It was considered necessary, therefore, to adopt a different type of procedure that would first separate the nitrate from the soil and then add it to the digest at the time of distillation. A number of methods were accordingly tried as the result of which it was found that addition of pure calcium sulphate (5 g.) followed by repeated leaching removed all the nitrate but practically no organic matter. Even added nitrates can be successfully removed in this manner. After removal of the nitrate, the soil was digested in the usual manner, and, after reduction with zinc, mixed with the extract containing nitrate and then distilled with excess of alkali and Devarda's alloy (0·5 g.). The results have been given in Table XII.

It was observed that even with 0·5 g. of Devarda's alloy there was considerable frothing and that the alkali tended to pass over with the spray. Addition of a small quantity of paraffin oil reduced the frothing. Metallic zinc was less violent in its action than Devarda's alloy though larger quantities (about 5 g.) had to be used to ensure complete reduction.

TABLE XII.

Description of soil	Total Nitrogen (p.p.m.) as estimated from			
	Soil alone (control)	Soil + nitrate (80 p.p.m. of N)	Soil + nitrate (160 p.p.m. of N)	Soil+nitrate (320 p.p.m. of N)
Travancore—Sandy loam, alkaline ..	217	294	370	530
South Bihar—Alluvial ..	361	435	515	677
Sholapur—Medium, black ..	415	492	570	730
Punjab—Irrigated rice land ..	596	668	750	908

As previously mentioned, most soils may not require any special treatment for the inclusion of nitrates in the estimate of total nitrogen. The more recent observations of Harihara Iyer and Rajagopalan (private communication) would indeed show that no nitric acid is lost during oxidative digestion with refluxing, so that there would appear to be no need for any pretreatment to include that form of nitrogen.

Comparative efficiencies of reduction in acid as well as in alkaline media.— As reduction in acid medium and cooling prior to distillation occupies some time, a number of trials were carried out combining reduction with zinc in alkaline medium together with distillation. The results showed, however, that while correct values were obtained in a few cases, comparatively low estimates were obtained in others. Moreover, when the method was extended to other biological materials such as yeast, leaf powder and seedcake, reduction in alkaline medium yielded lower and less consistent results than that in acid medium.

Estimation of nitrogen in urea.— When oxidative digestion was applied, as such, to urea, low and discordant values were obtained. Thus, when a number of parallel specimens were digested, values such as 39·7, 37·6, 40·0 and 38·5 per cents. were obtained instead of the expected value of 46·2 per cent. A study of the related literature showed that, under such conditions, a part of the nitrogen would be lost in the elementary form (Oechner De Coninck, 1899). It was thought probable, however, that by varying the conditions of digestion, such as proportion of acid to water and time of heating, it should be possible to avoid the loss of nitrogen. The results thus obtained have been presented in Table XIII.

Similar low estimates were obtained even when the proportion of acid to water was raised to 6 : 1 : nor was any improvement noticed when the

TABLE XIII.

Proportion of acid to water	Nitrogen per cent. found after digestion for			
	30 mins.	1 hr.	2 hrs.	3 hrs.
2 : 1 ..	38·6	37·8	46·2	38·9
4 : 1 ..	39·5	38·9	39·6	40·5
1 : 1 ..	40·6	39·8	41·2	40·0

solution of urea was pre-treated with alkali for varying periods of time (4–24 hrs.) prior to digestion.

In view of the above difficulty, some experiments were next carried out in which aqueous solutions of urea were boiled with only the acid. It was then found that when the proportion of acid to solution was as 2 : 1, the entire quantity of urea was digested in under half-an-hour.

The observations were then extended to determine whether total nitrogen contents of soils containing urea (and amides in general) can be accurately estimated. To specimens of three soils known quantities of urea were added. The mixtures were first boiled with 2 : 1 acid for 30 mins. and then subjected to oxidative digestion in the usual way. The results have been given in Table XIV.

TABLE XIV.

Soil from	Total Nitrogen (in mg.) as found in			
	Soil alone	Soil+urea (23·1 mg. of N)	Soil + urea (46·2 mg. of N)	Soil+urea (92·4 mg. of N)
Bangalore ..	5·66	28·8	52·0	98·0
Ranchi ..	4·88	28·0	51·0	97·4
Jaffna ..	3·25	26·2	49·5	95·75

It may be noted that very nearly correct results were obtained in all the cases.

The foregoing observations would suggest that if a soil is comparatively rich in free amides, accurate estimates of total nitrogen can be obtained only by pre-digesting soil for about 30 mins. with 2 : 1 acid prior to addition of the

oxidising agent. This would make the process rather long and tedious, so further experiments are in progress to devise a modification that will either eliminate the pre-boiling or, at any rate, reduce it to a minimum. Some encouraging results in this direction have already been obtained and will be reported in the next communication.

Estimation of nitrogen in cyanamide.—The difficulty in obtaining accurate estimates of nitrogen in cyanamide is well known. Richardson (1932) who made a special study of this problem has recommended boiling with comparatively dilute acid (acid : water 2 : 5) for 1–2 hrs. so as to ensure complete hydrolysis of the cyanamide prior to conducting the usual Kjeldahl digestion. Since urea is the immediate product of hydrolysis, it was first thought that adoption of the same procedure as in the previous experiment would yield correct estimates of nitrogen. Such was not, however, the case and, indeed, even longer boiling as suggested by Richardson followed by oxidative digestion failed to yield the correct results. Some further experiments were therefore carried out in which ferrous, ferric or manganous sulphate was added during the pre-boiling (with 2 : 1 acid) stage to catalyse hydrolysis. These yielded the best results and indeed, it was found that in the case of cyanamide alone, the entire digestion was complete after refluxing for about two hours. There was no need for any further oxidation (Table XV).

It may be seen from the above that, except in the last three cases, not only were the values low, but there was also no benefit derived through prolonged boiling. It has to be inferred that in most of those cases nitrogen was either lost from the system or present in some form which was not amenable to oxidative digestion.   •

When cyanamide (0·2 g.) was added to soil (10 g.) and pre-digested with acid (2 : 1) and manganous sulphate, it was observed that the digestion proceeded very rapidly. There was no need for prolonged boiling as in the case of cyanamide alone. Pre-digestion for 1 hr. followed by the usual oxidative digestion was sufficient to obtain correct estimates of total nitrogen.

The average soil does not contain any free cyanamide. Even if any is added as fertiliser, it is soon decomposed, so that the problem of having to include it in the estimate of total nitrogen rarely ever arises. In cases where cyanamide has been recently added, it can be easily detected and the procedure for the estimation of nitrogen modified accordingly.

A comprehensive procedure for the estimation of nitrogen in soils and biological media.—The material to be digested (soil, 10 g. ; others in smaller quantities) is weighed out into a large, flat or round bottom flask, preferably

TABLE XV.

Treatment	Total Nitrogen per cent, after digestion for		
	30 mins.	1 hour	2 hours
2 : 1 acid and $K_2Cr_2O_7$ added simultaneously ..	13·5	13·7	13·6
Refluxed with 2 : 1 acid for 30 mins. and $K_2Cr_2O_7$ then added	12·9	13·6	13·0
3 : 2 acid and $K_2Cr_2O_7$ added simultaneously ..	12·8	13·2	12·6
Refluxed with 3 : 2 acid for 30 mins. and $K_2Cr_2O_7$ then added	12·6	12·5	12·9
4 : 1 acid and $K_2Cr_2O_7$ added simultaneously ..	11·5	11·8	11·6
1 : 2 acid and $K_2Cr_2O_7$ added simultaneously ..	12·6	12·0	12·1
Overnight standing with 4 per cent. alkali followed by simultaneous addition of excess of acid and $K_2Cr_2O_7$	5·4	..	5·0
Do. $K_2Cr_2O_7$ added after pre-digestion with acid for 30 mins.	13·0	..	13·2
Refluxed with 2 : 1 acid and $Fe_2(SO_4)_3$ only ..	14·6	15·4	15·9
„ and $FeSO_4$ only	14·6	15·1	15·9
„ and $MnSO_4$ only	14·0	15·7	16·1

Value expected = 16·3 per cent.

the one to be used subsequently for distillation. Mercuric oxide or sulphate (about 2 g.) is then added and the mixture treated first with water (20 c.c.) and then with pure (N-free), concentrated sulphuric acid (10 c.c.). The flask is fitted with an air-cooled condenser (any piece of glass tube, fairly wide and about 2 feet long will suffice) and the contents raised to gentle boil. After boiling for 30 minutes, potassium dichromate (about 5 g.) is added to the mixture and the boiling continued for a further period of 30 mins. The flask is then taken out and the contents treated with pure, powdered zinc (5-7 g.) followed by dilution with water. This results in very vigorous evolution of hydrogen accompanied by reduction of excess of chromic acid and any nitric acid which may be present in the medium. Any nitrogen

present in combination with chromium is also released by this treatment. The mixture is then boiled for 15 minutes, during which period the added zinc is generally used up. The contents of the flask are then cooled, treated with excess of alkali and distilled in the usual way.

All specimens of zinc—including the purest analytical reagents—contain some nitrogen, so it may be necessary to conduct a blank determination whenever a new sample is taken. A convenient arrangement will be to take a fairly large stock of zinc (1–2 kg.) and to make one set of blank determinations on representative samples taken therefrom.

Estimation of nitrogen in some biological materials.—The foregoing procedure was next extended to other organic substances containing free or combined amide nitrogen. In all the cases, the materials to be digested were boiled with manganous sulphate and acid (2 : 1) for 30 mins. prior to addition of oxidising agent. The digestion was then continued for 30 mins. after which the digest was reduced with zinc and distilled with excess of alkali. The results which have been presented in Table XVI show that correct estimates were obtained in all the cases.

TABLE XVI.

Material	Nitrogen (per cent.) as estimated by	
	Kjeldahl ('wet') digestion	Pre-boiling with acid followed by oxidative digestion
Farmyard manure ..	0·64	0·67
Hongay leaf (dry) ..	3·42	3·40
Hongay seed-cake ..	4·48	4·45
Mahua seed-cake ..	2·57	2·48
Dried blood ..	12·53	12·49
Rubber latex—Sample I ..	0·35	0·36
Sample II ..	0·05	0·05

Experiments with other oxidising agents.—A few preliminary trials were carried out using chromic anhydride, permanganate and sodium bismuthate in acid as well as alkaline media. The results have been given in Table XVII.

TABLE XVII.

Treatment	Nitrogen in parts per million	
	Expected	Found
Soil (10 g.) +KMnO$_4$ (10 g.)+NaOH (20 c.c., 50 per cent.). Mixture distilled	630	223
Soil (10 g.) +CrO$_3$ (10 g.)+ NaOH (20 c.c., 50 per cent.). Mixture distilled	630	102
Soil (10 g.) +Sod. bismuthate (3 g.) + Water (20 c.c.) + H$_2$SO$_4$ (40 c.c.). Mixture digested and then distilled with excess of alkali	566	150
Soil (10 g.) +KMnO$_4$ (20 g.)+Water (20 c.c.)+H$_2$SO$_4$ (10 c.c.). Refluxed for 30 mins. and then distilled with excess of alkali	566	340
Do. but reduced with zinc prior to distillation with alkali	566	500
Soil (another sample ; 10 g.) + KMnO$_4$ (20 g.) +Water (20 c.c.)+H$_2$SO$_4$ (40 c.c.). Refluxed for 30 mins. and then distilled with excess of alkali	630	426
Do. but reduced with zinc prior to distillation with alkali	630	581

It was observed that when the soil was distilled as such in alkaline media, there was very slow distillation of ammonia. Even prolonged heating yielded only less than a third of the expected value for nitrogen. Both the bismuthate and the permanganate tended to decompose rapidly in presence of the fairly concentrated acid that was employed. The low values obtained, especially in the case of the former, were largely due to this fact. There was evidence of partial oxidation to nitrate, as also of retention in other forms, in the case of specimens digested with acid permanganate. Further work with more dilute acid solutions and with other oxidising agents is in progress and will be reported in a subsequent communication.

Discussion.

The present enquiry has led to a number of findings of much scientific interest. In addition to providing a simple method for the estimation of nitrogen in soils and biological materials, it has also thrown much light on the nature of the changes attendant on the oxidative digestion of nitrogen.

The advantages of the new method have already been enumerated. As the procedure is also comparatively simple, it may be reasonably expected that, before long, it will be adopted in routine practice. A few improvements are, nevertheless, needed. Boiling with the reducing agent in acid medium takes some time and should, if possible, be replaced by some treatment than can be combined with the distillation. A further problem is the blank for the reducing agent which, unfortunately, is highly variable. Even the purest preparations of metals contain some nitrogen, so it may be desirable to reduce the quantity of such agents to the barest possible minimum.

The products of oxidative digestion are more varied than those obtained by the Kjeldahl method. The latter forms exclusively ammonium sulphate whereas the former produces a few other substances as well. During Kjeldahl digestion, there is no loss of nitrogen except that of nitric acid and few other volatile, non-digestible forms which may be originally present, but during oxidative digestion, there is some danger of loss if the conditions are not adequately controlled. There is also the production of nitric acid and certain other complex forms which have to be first reduced if accurate estimates are to be obtained. It is difficult to state whether any nitrogen is mechanically retained under such conditions. Although the new procedure includes all the forms of nitrogen in the estimate, it would, nevertheless, be of much interest to obtain further information regarding the mechanism of the related processes. Such knowledge will also facilitate further simplification of the method of estimating nitrogen.

The difficulties encountered with urea and cyanamide have shown the necessity for modifying the procedure in a few cases. Pre-boiling with acid takes some time and when combined with the oxidative digestion, the process becomes somewhat slow and tedious. There is scope for improvement in this direction and, as previously mentioned, some promising results have already been obtained.

The use of other oxidising agents, though not so far promising, may open out a highly useful field of research. It is probable that some of them may function at much lower concentrations of acid than those required in the case of chromic acid: they may also yield colourless digests either by themselves or after reduction. This would be of great advantage as it would provide a useful check on the progress of digestion.

Summary.

1.   When distilled with excess of alkali, the residue left after wet combustion of soil yielded lower estimates of nitrogen than those obtained by either the official Kjeldahl method or by 'wet' digestion.

2. Prolonged heating of the chromic acid digest led to no appreciable improvement in the estimate of nitrogen : nor was any increase obtained by further 'wet' digestion of the insoluble residue.

3. Kjeldahl digestion with chromium sulphate (in place of potassium sulphate) led to reduced estimates of total nitrogen being obtained. The values were also discordant.

4. Pre-treatment of soil with water did not help to improve the estimate of nitrogen obtained by oxidative digestion. Neither addition of salts of certain heavy metals during digestion nor treatment with certain chemical precipitants before distillation with alkali led to any increase in the value for total nitrogen.

5. Boiling the acid digest with the reducing agents led to marked improvement in the estimate of nitrogen. The best results were obtained with zinc. (All specimens of zinc contain some nitrogen, so some correction must be applied for the ammonia derived from that source.)

6. Some soils yield accurate estimates of total nitrogen even if they are boiled with the oxidising mixture in open Kjeldahl flasks. Others have to be refluxed under air- or water-cooled condensers. A convenient procedure will be to reflux the digest in all the cases and reduce the digest prior to distillation.

7. The mechanism of the action of zinc has been discussed and shown to be largely one of reduction.

8. When the proportion of acid to water was as 1 . 1 or less, the progress of digestion was slow. When the ratio was raised to 2 : 1, it was complete in under 30 minutes. The rate of digestion was not appreciably improved by further increase in the proportion of acid.

9. The presence of chlorides led to marked decrease in the estimate of total nitrogen. This was traced to the formation of chlorine which reacts with ammonia and amides forming elementary nitrogen. The production of chlorine was avoided and correct estimates of total nitrogen obtained by addition of small amounts of mercuric oxide or sulphate to the digesting mixture. The mercury salts in the digest tend to retain some nitrogen but that can be released by boiling with zinc or reduced iron.

10. Based on the above and allied observations, a general method of oxidative digestion applicable to all types of soils has been developed. The procedure for adoption in routine practice has been described. Close agreement has been found between the results obtained by the new method and that determined by Kjeldahl ('wet') digestion.

11. When the soil contains considerable amounts of nitrate, the latter

will have to be reduced with dilute alkali and Devarda's alloy prior to oxidative digestion. An alternative procedure will be to treat the soil with calcium sulphate and water and repeatedly leach out the clear supernatant into the distilling flask. The residue is then subjected to oxidative digestion and the digest, along with the leachate previously obtained, reduced with zinc prior to distillation with alkali.

12. Reduction with zinc in acid medium is more effective than that in alkaline medium. Aluminium or Devarda's alloy is fairly effective in alkaline media, but there is always the danger of alkali being mechanically carried over, thus necessitating re-distillation.

13. Oxidative digestion of urea led invariably to low estimates of total nitrogen being obtained. Neither increase in the proportion of acid to water nor prolonged heating led to any improvement in the estimate. On the other hand, pre-digestion for about 30 minutes with 2 : 1 acid alone was sufficient to digest all the urea. This, combined with oxidative digestion, led to accurate values of total nitrogen in soils containing known amounts of urea.

14. Cyanamide also offered some difficulties, but digestion with 2 : 1 acid containing manganous ˙sulphate hastened the digestion and yielded accurate results. When mixed with soil, the digestion of cyanamide was complete in under one hour.

15. The modified procedure was adopted for the estimation of nitrogen in some biological materials. There was generally close agreement between the values thus obtained and those determined by the Kjeldahl method.

16. The possibility of using other oxidising agents in place of chromic acid has been indicated.

17. Attention has been drawn to the need for further simplification of procedure for oxidative digestion. Certain promising lines of future research have been indicated.

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CONTENTS

SECTION A

FLUORESCENCE IN CYCLOHEXANE.

By R. Padmanabhan.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. *Introduction.*

WHILE investigating the polarisation of light scattering in cyclohexane for different wavelengths an intense continuous spectrum in the region 2700–4000 Å was found to have developed after long exposures which interfered with the depolarisation measurements. A study of this fluorescence was therefore taken up in order to ascertain its cause so that it could be avoided if possible. It was found that Haberl[1] had noticed this phenomenon a few months earlier and had arrived at certain conclusions regarding the nature and origin of the fluorescence in cyclohexane. Since the observations of the author do not agree with those of Haberl and lead to entirely different results it has been thought worthwhile to publish them.

2. *Experimental.*

The apparatus used for the investigation was made completely of silica and consisted of a distilling flask to which a tube closed at both ends was attached. The requisite amount of pure cyclohexane was introduced into the apparatus which was then evacuated and sealed off. After repeated distillations the pure dust-free liquid which collected in the tube was used for the work. The light from a quartz mercury point source lamp was focussed on to its side with a quarter condenser and the light scattered at right angles was examined with a quartz spectrograph (dispersion: 10 Å per m.m. at $\lambda = 4000$ Å).

3. *Preparation of Pure Cyclohexane.*

250 gms. of cyclohexane (T. Schuchardt) labelled as "pure" were agitated thoroughly with oleum for 10 hours on a shaking machine, washed repeatedly with a dilute suspension of precipitated chalk and then with distilled water after which it was distilled twice over anhydrous calcium chloride in an all-glass apparatus using a Widmer-Schenck column for fractionation. The boiling point was steady at 77° (pressure = 680 m.m.). The middle fraction was used for the experiment.

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[1] *Ann. der Physik.*, 1934, **21**, 301.

1. *Nature of the Fluorescence Spectrum.*

According to Haberl (*loc. cit.*) the fluorescence band has two maxima at λ ≈2900 Å and 4000 Å respectively, the latter being nearly as intense as the scattered 4046 line of mercury. The phenomenon, according to him, is constant and reproducible, while long irradiation with a mercury arc has no influence. The present observations, however, point to a wholly different conclusion. For example, it was noticed at the outset that, under identical conditions, such as time of exposure, intensity of incident light, etc., the fluorescence spectrum varied from experiment to experiment. At first only a feeble continuous spectrum from 2700-2900 Å was observed but with successive exposures the intensity increased while the spectrum gradually extended towards the side of longer wavelengths. After illuminating the substance for 8 hours with a quartz mercury arc, it was noticed that the light track, barely perceptible before, was now clearly visible. The fluorescence band was also found to have extended into the visible spectrum and although in the absence of a microphotometric record the exact positions of the maxima could not be located, there appeared to be a rough agreement with the results of Haberl. It appears therefore that his picture of the fluorescence spectrum has been obtained with a sample which had been strongly illuminated with ultraviolet light. His experimental arrangement in which a large mercury arc is placed alongside the tube containing the substance[2] is quite in contrast to the feeble mercury point source (about 2 amperes at 40 volts) used in the present work and supports such a conclusion. The strong fluorescence in the irradiated substance was found to be completely removed by distilling it twice or thrice indicating thereby that the constituents responsible for the fluorescence are non-volatile.

5. *Is Fluorescence a Characteristic of Pure Cyclohexane?*

The above observations suggest that, on exposure to the ultraviolet, some photochemical decomposition takes place and the products of decomposition give rise to the observed fluorescence. This is further illustrated by the following experiment: Two pictures of the fluorescence spectrum were taken on a single plate, the first with an exposure time of one hour while the second was taken with four exposures of 15 minutes each, the liquid being distilled afresh after every exposure. The intensity of the fluorescence was distinctly greater in the former case. In order to decide definitely whether the fluorescence could be wholly ascribed to the products of photochemical action, an apparatus was designed (Fig. 1) in which the

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2 Haberl, *Ann. der Physik.*, 1934, **21**, 301.
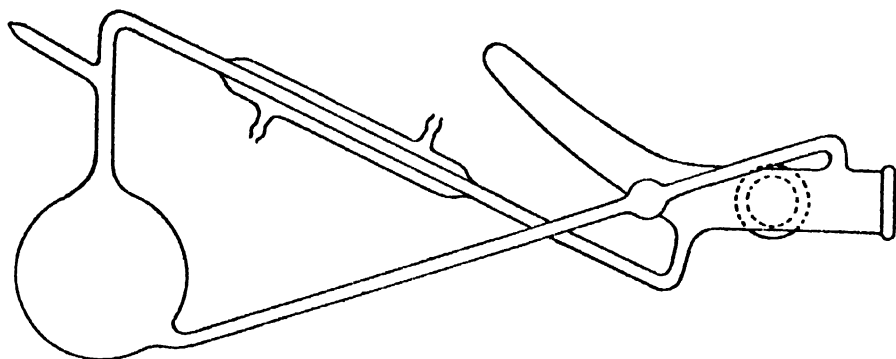
FIG. 1.

Apparatus for Continuous Distillation.

substance could be continuously renewed by distillation. The arrangement of Pal and Sen Gupta[3] was first tried but was not found to work satisfactorily on account of two reasons: (1) The inlet and outlet tubes are both situated on the upper side and hence only the top layer of the liquid is renewed, the main bulk of the liquid remaining unchanged. (2) The distilling flask being at a lower level, the liquid siphons out intermittently from the tube. In the apparatus illustrated above, these difficulties have been avoided (1) by placing the inlet on the under surface of the tube and (2) by blowing a small bulb on the outlet tube so as to avoid the siphoning action. If the distilling vessel is kept immersed in hot water, liquid enters and flows out of the tube in a continuous stream. The apparatus was made of pyrex glass except for the two quartz windows which were stuck on with a solution of sodium silicate. It was found that, with this arrangement, even an exposure of 6 hours, did not show any continuous spectrum on the plate (Fig. 2). A picture taken without the substance being renewed by continuous distillation is also shown side by side for comparison. The contrast is clearly noticeable. This experiment furnishes definite proof that pure cyclohexane has no fluorescence at all.

6. *Origin of the Fluorescence.*

It became of interest now to examine what wavelengths are photochemically active in the above reaction. According to Haberl, the interposition of a thin glass plate in the path of the incident light which cuts off all wavelengths less than $\lambda = 3000 \text{Å}$ removes the fluorescence completely. He concludes therefrom that the resonance line $\lambda = 2537 \text{Å}$ is perhaps responsible for the fluorescence. A picture was taken with a filter of 1 cm.

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[3] *Ind. Jour. Phys.*, 1930, **5**, 609.

thickness of carbon tetrachloride to cut off all wavelengths less than $\lambda =$ 2600 Å in the incident light. The picture obtained was quite free from any continuous spectrum, suggesting thereby that only wavelengths less than $\lambda = 2600$ Å were responsible for the decomposition.* A similar experiment, however, using a 5 m.m. thickness of 2 molal solution of acetic acid as filter which absorbs all wavelengths transmitted by quartz but less than 2400 Å[4] shows the fluorescent spectrum with almost undiminished intensity. Since the resonance line $\lambda = 2537$ Å is by far the strongest line of the mercury arc in the region 2400-2600 Å this wavelength seems to be largely responsible for exciting the fluorescence spectrum.

Further work on the nature of the photochemical reaction is in progress.

In conclusion, the author wishes to express his grateful thanks to Sir C. V. Raman, Kt., F.R.S., N.L., for his valuable help and guidance during the progress of this investigation.

Summary.

(1) An apparatus for continuous distillation has been described which works satisfactorily and is free from the defects of the arrangement described by Pal and Sen Gupta.[5]

(2) With the help of the above apparatus pure cyclohexane is shown to have no fluorescence; that reported by Haberl[6] has been proved to be due to products of photochemical decomposition.

(3) The wavelengths in the region $\lambda = 2400-2600$ Å have been found to be active in the above reaction.

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* Contrary to the observations of Haberl, in the case of cyclohexane which had been exposed to the mercury arc for some time, the fluorescence in the visible is not removed by introducing a glass plate in the path of the incident light. The intensity of the fluorescence, however, is somewhat diminished as can be seen from the photographs (Figs. 3 and 4). This shows the necessity for distinguishing between wavelengths responsible for photochemical action and wavelengths capable of exciting fluorescence.

4 Kishakowsky and Nelles, *Phys. Rev.*, 1932, **41**, 595.

5 *Ind. Jour. Phys.*, 1930, **5**, 609.

6 *Ann. der Phys.*, 1934, **21**, 301.
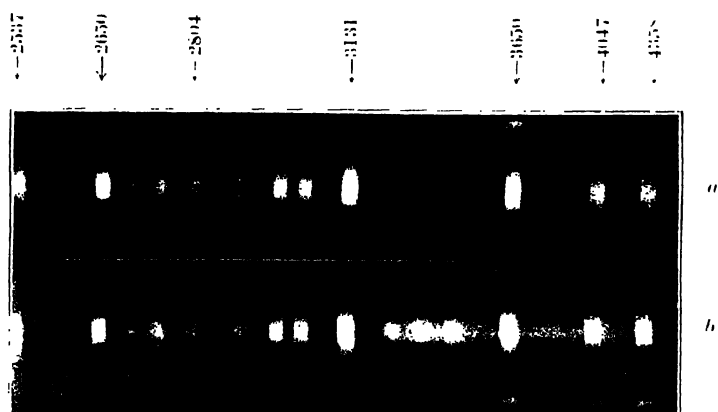
FIG. 2.

(a) With continuous distillation.
(b) Without continuous distillation.
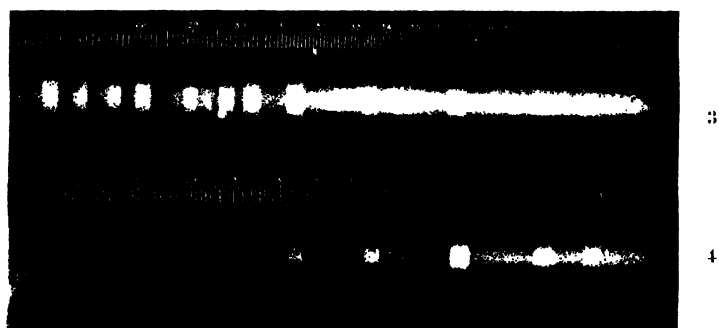
FIG. 3.   Fluorescence spectrum of irradiated cyclohexane, exposure
         2 hours.

FIG. 4.   Fluorescence spectrum with glass plate in the path of the incident
         light, exposure 2 hours.

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THE DENSITY AND COMPRESSIBILITY OF SILICANE AND SILICOETHANE.

By K. L. Ramaswamy

AND

G. Gundu Rao.

(From the Department of General Chemistry, Indian Institute of Science, Bangalore.)

In connection with work previously published on the dielectric coefficients of gases,[1] approximate measurements of compressibility at different temperatures were required. Density determinations for the purpose of checking the purity of the gases were also desirable and consequently the apparatus now to be described was erected, the design being due to Dr. H. E. Watson.

Among the gases examined were silicane and silicoethane, the densities of which appear to have been measured only by Stock and Somieski,[2] who gave the values 32·29 and 63·8 or 64·2, as compared with O_2 32. No attempt was made by these authors to deduce the atomic weight of silicon as the compressibilities were not determined. Silicane is a gas which can be prepared in a state of considerable purity without great difficulty and the results of the present series of experiments are recorded as indicating that, with a few additional refinements, the density method would be suitable for an accurate determination of the atomic weight of silicon.

Apparatus.

The essential features of the apparatus consisted of a density bulb which could be detached and weighed, and a second bulb of known volume into which the gas could expand, so that the pressure of a given quantity could be determined at two different volumes. Details are shown in Fig. 1. A is the density bulb made of pyrex glass with a long calibrated capillary stem closed by the tap T and fitted with a ground conical end. The joint was made gas-tight with a very little apiezon grease, which did not appear to be attacked by the gas. B is a small bulb to condense the gases and fractionate them when necessary. M is a cylindrical bulb with its ends made from two pieces of the same tubing from which the manometer, M_1,

[1] *Proc. Roy. Soc.,* 1934, **143A**, 558.

[2] *Ber.,* 1916, **49**, 1, 111.

is constructed. In these ends are fixed two glass points, P and P_1, to which, the mercury meniscus can be set. The volume of M is approximately the
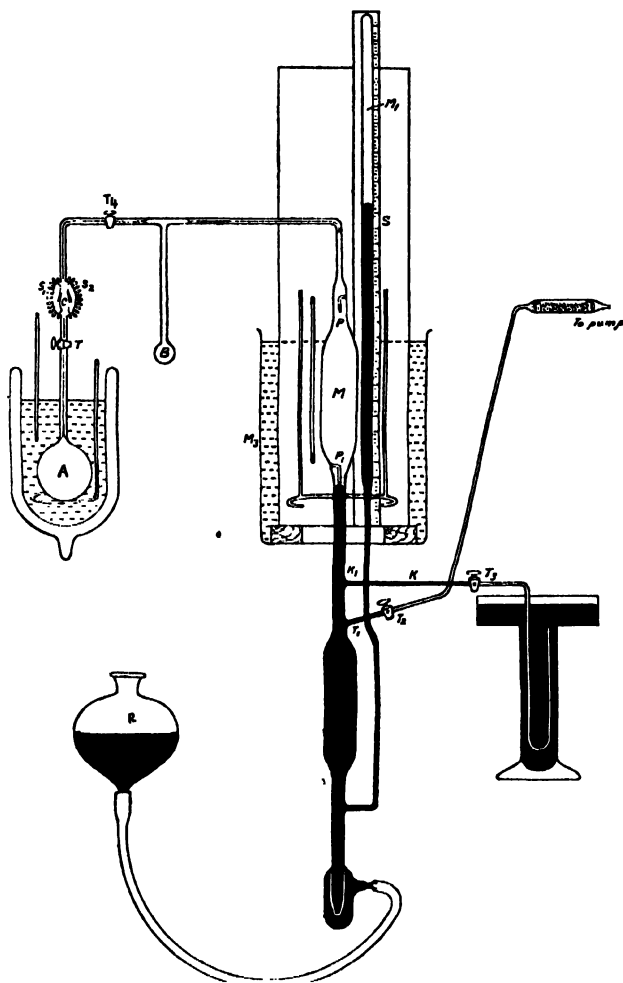
Fig. 1.

same as that of A. At T_1, a side tube with a tap T_2 connects the apparatus to a Toepler pump. Gas can be introduced through the syphon K and tap T_3.

All the apparatus except the density bulb was made of durosil glass. M was surrounded with a glass water jacket and the manometer was enclosed in a box with plate glass sides to protect it from draughts.

Calibrations.

The calibration of the apparatus was done before assembly. The volume of the density bulb A and the change with temperature were determined, using air-free distilled water. The change of volume with pressure was found by noting the increase in the height of level of water in the capillary stem by a known decrease of pressure.

A pyrex counterpoise was constructed with the same weight and external volume as the density bulb. These were checked by finding the loss of weight in water.

The volume of M between the points $P \ldots P_1$ was determined by attaching a tap at the bottom and weighing the amount of distilled mercury required to fill it and the dead space and other parts of the apparatus were calibrated in a similar manner. The thermometers, thermocouples and the weights were all calibrated in the usual way.

All pressure readings were reduced to $0°$ and $45°$ lat. at sea level and corrected for meniscus height. The glass scale by the Société Genevois was checked against a standard invar scale.

Experimental Procedure.

After attaching the density bulb to the apparatus as shown in Fig. 1, the entire apparatus was evacuated thoroughly. The gas was then introduced and fractionated if necessary. When measurements had to be made at ordinary temperatures, the baths for the density bulb and the bulb M were adjusted to the same temperature as that of the room. At other temperatures, the necessary low-temperature bath was used only for the density bulb A. Sufficient time was allowed for equilibrium to be attained. A thin layer of paraffin oil was spread over the surface of the water in M_3 to minimise the loss of heat due to evaporation.

When the pressure was steady, the level of mercury was adjusted to the pointer P_1 with the meniscus rising. The pressure, meniscus heights, the temperature of the dead space and the temperatures of the two baths were then read. Next, the level of the mercury was adjusted to the pointer P and similar readings were taken. This was repeated several times.

Before measuring the density, it was found advisable to test the gas for traces of hydrogen by cooling B in liquid air and evacuating. After the removal of any trace of hydrogen present, B was placed in water at room temperature and the temperatures and pressure read as soon as they were steady. The tap T was then closed, the gas in the dead space was condensed in the bulb B and the tap T_4 was closed.

The bulb was removed and the grease washed off with ether. After drying, it was placed in the balance along with the counterpoise, which had been subjected to similar treatment. Several hours were allowed to elapse before weighings were made. As in the case of the calibration of weights, the interchange method was employed when weighing the gases, the sensitiveness being determined in each case. The weight of the empty bulb was taken both before and after the experiments, and it was found that no measurable change occurred. This indicated that the gas did not decompose in the interval.

The necessary corrections for the weight, volume, pressure and temperature were applied and the density calculated.

Compressibility.

The apparatus may be considered to consist of three parts, (1) the density bulb, (2) the dead space, (3) the expansion bulb M. If it be assumed that the compressibility is linear, $i.e.$, $pv - p_o\, v_o\, (1 - Ap)$, the mass of gas in a volume v, at a pressure p, is proportional to $\dfrac{pv}{(1-Ap)T}$, where T is the absolute temperature.

Denoting by suffixes the volumes and temperatures of the three parts, and, by a dash, corresponding quantities at a second observation, the general equation—

$$\frac{pv_1}{(1-A_1p)T_1} + \frac{pv_2}{(1-A_2p)T_2} + \frac{pv_3}{(1-A_3p)T_3}$$
$$= \frac{p'v'_1}{(1-A'_1p')T'_1} + \frac{p'v'_2}{(1-A'_2p')T'_2} + \frac{p'v'_3}{(1-A'_3p')T'_3}$$

holds good, since the pressure throughout the apparatus is constant. In practice, this admits of considerable simplification : v_3 is small and equal to v'_2, while T_2, T_3, T'_2 and T'_3 are very nearly equal so that A_2, A_3, A'_2 A'_3 may be considered equal.

When the compressibility is determined at room temperature in the manner already described, p is the pressure with the mercury at the point P_1, and p' when it is raised to P. v'_3 is consequently zero ; the temperatures are all nearly the same and all the A terms are equal to, say, A_T. A_T may, therefore, be determined, all the other quantities being known. On repeating the experiment with the bulb cooled, T_1 and T'_1 are unequal and, consequently, so are A_1 and A'_1—say, A'_T. Since A_T is now known, A'_T may be calculated.

The compressibility at low temperature may also be determined by raising the mercury to the upper point and then cooling the density bulb

and reducing the pressure simultaneously to keep the volume constant. This method has the advantage that v_3, v'_3 are both zero and hence the result is not affected by a calibration error in this part of the apparatus ; on the other hand, the temperature T'_1 must be known with greater accuracy than in the other method.

Preparation and Purification of Materials.

The silicon hydrides were prepared by the method recommended by Stock and Somieski.[3] Magnesium silicide was made by the ignition of a mixture of anhydrous silica free from alkali, and magnesium turnings free from arsenic and phosphorus, Kahlbaum's analytical reagents being used for the purpose. The silicide was then powdered, sieved and the excess of un-burnt magnesium removed mechanically. About 25 grams of this product were then decomposed in an atmosphere of hydrogen by adding it gradually to a ten per cent. solution of pure hydrochloric acid, the temperature being kept in the neighbourhood of 50° C.

The evolved gas after passing through a reflux condenser, a spray trap and a U tube at $-10°$ C., was condensed in a U tube cooled by liquid air and the major portion later boiled off into a gas holder. The gas was recondensed and pumped off at a temperature of about - 155° C., the vapour pressure being 30 millimetres. At this temperature the vapour pressure of Si_2H_6 according to Stock is less than 0·1 mm. As soon as the pressure fell to 1--2 mm., the bath was replaced by one of solid ether (- 115°) and pumping continued until the pressure reached 2 mm. which is just above the vapour pressure of Si_2H_6 at that temperature. On raising the tempera-ture to -90° C. more gas was removed consisting mainly of Si_2H_6. This fractionation was repeated several times until the gases appeared fairly pure.

Preliminary density measurements gave high values for SiH_4 and these were ascribed to the presence of carbon dioxide. This was removed by inserting into each tube containing the gas a small piece of freshly prepared quicklime which did not attack the silicane at an appreciable rate. The gas was then fractionated once more before making measurements.

Results.

The following compressibility values were obtained, each being the mean of two observations. Owing to an unfortunate accident in which most of the gas was lost, it was not possible to make measurements with Si_2H_6 at low temperatures. These values although sufficient for the purpose for which they were originally required are not accurate enough for atomic

[3] *Ber.*, 1916, **49**, 111.

TABLE I.

	$t°$C.	$A \times 10^3$	$t°$C.	$A \times 10^3$	
SiH$_4$ ..	27·0	7·1	24·0	8·7	
	27·0	8·4	27·5	7·9	Mean 8·4
	25·0	9·2	26·0	8·9	
·	−79·8	26*	−79·8	20	
	,,	21*	,,	21	Mean 23
Si$_2$H$_6$ ..	28·0	25	27·0	26	
·	26·5	26	27·0	27	Mean 26

* Constant volume method.

TABLE II.

Gas	$t°$C.	V c.c.	W grams	P mm.	D$_{N.T.P.}$ (Uncorr.)	D$_{P=0}$	Mol. wt.
Oxygen ..	23°·60	106·64	0·10710	581·02	1·4312	1·4302	
	24°·83	,,	0·12537	681·30	1·4307	1·4296	
Monosilane	23°·56	,,	0·12386	663·84	1·4441	1·4338	32·09
	24°·58	,,	0·12904	691·95	1·4487	1·4376	32·17
	24°·26	,,	0·12985	696·63	1·4477	1·4366	32·15
	25°·20	,,	0·12837	690·17	1·4480	1·4370	32·16
	25°·22	,,	0·12916	695·38	1·4460	1·4349	32·11
	−79°·8	106·53	0·12395	430·07	*1·4554*	*1·4348*	32·11
	,,	,,	0·12102	430·04	*1·4563*	*1·4357*	32·13
Disilane ..	28°·03	106·64	0·21312	585·16	2·8621	2·8050	62·77
	26°·95	,,	0·24819	677·50	2·8685	2·8021	62·71

Mean molecular weight of monosilane = 32·13

,,   ,,   disilane   = 62·74

weight determinations. They could probably be improved by reading the pressures with a micrometer eye-piece instead of the simple telescope actually used, and by jacketting the manometer.

The density values are given in Table II as well as two measurements with oxygen for comparison. The values for this gas are higher than the accepted value for the normal litre 1·4290, indicating a constant error. If, however, these are taken as standard, the resulting figures for the silicanes should be fairly accurate. In the column $D_p=0$ is given the reduced density or the weight of a normal litre if the compressibility were zero. These figures are calculated from the measured density and pressure and the compressibility. The molecular weight in the last column is obtained by multiplying 32·00 by the ratio of this reduced density to that of the mean value for oxygen 1·4299.

Discussion.

Stock and Kuss[4] made some preliminary determinations of the atomic weight of silicon by the decomposition of silane with sodium hydroxide and obtained a value 28·15. Although this value was far from being decisive it suggested that the then accepted value 28·30 was rather high.

A little later Stock and Somieski[5] measured the gas densities for silicane and silicoethane and obtained the values 32·29 and 63·8 or 61·2 for the molecular weights. Apparently no corrections for the deviations from Boyle's law seem to have been applied. Assuming the values of compressibilities obtained in the present investigations to hold at the temperatures of their measurements, the values for the molecular weights work out to be 32·02 and 62·3 for mono and disilanes respectively.

Baxter and co-workers[6] from the analysis of silicon tetrachloride and tetrabromide purified by thorough fractional distillation under exclusion of air and varying pressures, obtained as the mean of a large number of determinations the value 28·063 for the atomic weight of silicon.

An year later O. Hönigschmid and M. Steinheil[7] employing the same tetrachloride method arrived at the value 28·105 ± ·003. At present Baxter's value has been accepted, although the relatively higher value of Hönigschmid has no reason to be rejected, being the mean of sufficiently concordant results.

[4] *Ber.,* 1914, **47**, 3115.

[5] *Ber.,* 1916, **49**, 1, 111.

[6] *Proc. Amer. Acad. Arts and Sci.,* 1923, **58**, 245.

[7] *Zeit. anorg. Chem.,* 1924, **141**, 101.

The value obtained in the present investigations taking into consideration only the monosilane, comes out to be $28 \cdot 10 \pm \cdot 03$. This agrees practically with the value $28 \cdot 105$ given by Hönigschmid by an altogether different method. The value $28 \cdot 34$ calculated from silicoethane is undoubtedly too high owing probably to the impurities of the higher homologues and adsorption effects.

It is unnecessary to mention the several advantages of the gas density method over the other methods. It is interesting to note that the corrected molecular weights at the ordinary and low temperatures are practically the same, indicating thereby that no association occurs at low temperatures and also that the effects of adsorption of the monosilane are not appreciable.

Summary and Conclusion.

1. An apparatus for the simultaneous measurements of densities and approximate compressibilities at the ordinary and low temperatures, has been described.

2. Measurements of densities and compressibilities on monosilane at different temperatures and on disilane at the ordinary temperature have been made.

3. The atomic weight of silicon obtained from the corrected molecular weights obtained in the present investigations, has been compared with the existing values by other authors.

In conclusion we wish to express our grateful thanks to Prof. H. E. Watson for his valuable criticisms and constant interest in the work.

MOLECULAR CLUSTERING IN BINARY LIQUID MIXTURES.

(Variation with Composition and Temperature.)

BY R. S. KRISHNAN.

(From the Department of Physics, Indian Institute of Science, Bangalore.)

1. *Introduction.*

IT is well known that when certain pairs of liquids are brought together in suitable proportions, two liquid phases result ; for example, in the case of phenol and water, the upper phase is a solution of phenol in water and the lower phase a solution of water in phenol. As the temperature is slowly raised, the compositions of the two phases tend to equality until at a parti- cular temperature (called the critical solution temperature), the line of separation disappears and the whole mass presents the appearance of a single homogeneous phase. At this temperature the mixture exhibits a very marked turbidity or opalescence. The intensity of opalescence is markedly a function of temperature, being greatest at the critical solution temperature and becoming very weak as the temperature is removed from it in either direction. The earlier investigators in this field suggested as an explanation of the opalescence that the liquid mixture in the vicinity of the critical solu- tion temperature behaves as an emulsoid. Smoluchowski was the first to propose a thermodynamic theory of the phenomenon which later was elabo- rated by Einstein. Einstein's formula for the intensity of opalescence received experimental support from the quantitative studies of Fürth and Zernike. There are certain features of the phenomenon, however, that do not find an explanation on the basis of the Smoluchowski-Einstein theory, one of which is that the depolarisation of the opalescence remains finite instead of tending to zero as the critical solution temperature is approached. In a recent paper[1] in these *Proceedings*, the present author presented evidence that large molecular clusters exist in such liquid mixtures in the neighbour- hood of the critical solution temperature. The finite value of the depolar- isation of the opalescent light arises from the fact that the size of the clusters is not small in comparison with the wave-length of light. The existence of such clusters could be demonstrated by the simple optical method developed

[1] R. S. Krishnan, *Proc. Ind. Acad. Sci.*, 1934, **1A**, 211.

by the author. This consists in examining through a double-image prism, the light scattered in the transverse horizontal direction by the mixture when it is illumined with light polarised with electric vector horizontal. If clusters of size comparable with the wave-length of light are present, the horizontal component of the scattered light would be distinctly brighter than the vertical component. In a more recent paper[2] the author has reported the results of a detailed study of the opalescence of a series of binary mixtures over a wide range of temperature above the critical point. These results fully confirm the author's preliminary report, and demonstrate the existence of clusters in liquid mixtures—not only at the critical solution temperature but also at temperatures considerably removed from it. In the present investigation, a comparative study has been made of the intensity and depolarisation of the light scattered by mixtures of phenol and water in different proportions and at different temperatures, with a view to ascertain the influence of the composition and of the temperature of the mixture on the formation of clusters. The experimental results have an important bearing on other physical properties of the mixture, such as viscosity, magnetic birefringence, etc.

2. Experimental Details.

For the study of the effect of composition on the formation of clusters, mixtures of phenol and water were chosen as the most suitable, as it had been found from the previous investigation that the phenomenon is most pronounced in this case. Pure crystallised phenol was melted and was mixed with pure double distilled water in the requisite proportion, and the mixture was directly transferred to a clean and dry double bulb. The double bulb was then exhausted and sealed. The mixture was then got dust-free in one of the bulbs by repeated slow distillation and washing back into the other bulb. The bulb containing the dust-free mixture was then sealed off from the other. Six different mixtures of phenol and water containing respectively (a) 15% by weight of phenol, (b) 28% of phenol, (c) 34% of phenol, (d) 50% of phenol, (e) 60% of phenol and (f) 70% of phenol, were prepared dust-free in the manner indicated. The bulbs containing these mixtures were approximately of the same capacity and the quantity of mixture contained in each was also the same.

3. Measurement of the Depolarisation of the Scattered Light.

The experimental arrangement employed in the present investigation was similar to that previously employed[*] for the measurement of the

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[2] R. S. Krishnan, *Ibid.*, 915.

[*] *Loc. cit.*

depolarisations ρ_u, ρ_v and ρ_h with the incident light respectively unpolarised, polarised with the electric vector vertical and with the electric vector horizontal. The ordinary lens of long focus used for condensing the light was replaced by a photographic lens of the same focal length provided with an iris diaphragm. The bulb containing the particular mixture to be studied was kept immersed in the water-bath. The temperature of the water in the bath was slowly raised and was kept steady at about 30° above the temperature of complete miscibility. The mixture was properly shaken. The depolarisations ρ_u, ρ_v and ρ_h were measured by the usual method of Cornu, with the help of a double-image prism and nicol mounted on a stand so as to be capable of independent rotation about the same axis. The mixture was then slowly cooled down and the observations were repeated for a series of temperatures up to the critical point at which the mixture separated into two layers. The errors in the depolarisation measurements arising from the background illumination were avoided by viewing the two components of the scattered light against the same background. The error due to the finite convergence of the incident beam was almost negligible since the angle of convergence was less than 3°. The values of ρ_u, ρ_v and ρ_h for various mixtures are tabulated below.

4. *Intensity Measurements.*

The method usually adopted by the earlier investigators in this field for the comparison and measurement of intensities of scattered light was visual photometry. Although visual photometry has been recognised as one of the accurate methods for the comparison of intensities, it is defective in that the process is tedious and at the same time subjective in nature. The advantages of using a photocell together with a direct current amplifier for comparison and measurement of feeble intensities, *e.g.*, that of light scattered by a fluid, have already been pointed out by Mr. R. Ananthakrishnan.[3] The convenience of using a photocell in such experiments lies in the fact that the state of polarisation of the light has no influence on the photoelectric current which is a direct measure of the energy of light falling on the cell. Moreover, in contrast with the visual method it is objective in nature and is less tedious. In the present investigation, therefore, the photoelectric method was employed for comparing the intensities of light scattered by the mixtures of phenol and water at various temperatures.

The experimental arrangement used for the measurement of intensity was similar to that employed for the depolarisation measurements. But the following alterations were made. The nicol and double-image prism

[3] R. Ananthakrishnan, *Proc. Ind. Acad. Sci.*, 1934, **1A**, 201.

were removed from the observation side. The transversely scattered light
after passing through the side tube attached to the wooden box which con-
tained the scattering substance was allowed to fall on the V-shaped plate of
the Osram CMG 8 (gas-filled) cell manufactured by the General Electric
Company, Ltd., of England. The photocell was suitably mounted inside
a wooden case (well insulated) which was painted dull black. The leading
tube through which the scattered light passed, was also painted dull black
inside and projected into the wooden case of the photocell. The distance
of the photocell from the scattering substance was about nine inches. The
scattered light entering the cell was limited by a series of apertures. The
whole arrangement was such that when the mixture was illumined, only the
light scattered in the exact transverse horizontal direction entered the cell.

The photocell was connected to the Valve Bridge Amplifier as shown
in Fig. 1.
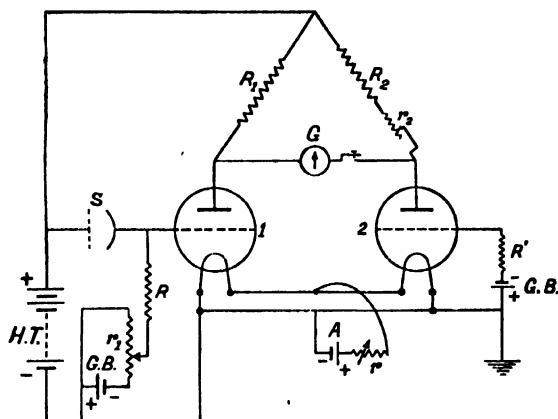
FIG. 1.

1, 2	..	L610 Marconi Dull Emitter Valves.
R_1, R_2	..	10000 ohm resistances.
R, R'	..	20 meg ohm grid leaks.
r_1	..	Varley Potentiometer (1200 ohms).
r_2	..	Adjustable rheostat (400 ohms).
r	..	Filament rheostat (5 ohms).
A	..	Filament battery (6 volts).
H. T.	..	High tension battery (115 volts).
G. B.	..	Grid bias battery (2 volts).
S	..	Osram CMG8 Photocell.
G	..	Microammeter.

This amplifier was originally set up by Mr. R. Ananthakrishnan to whom
the author's thanks are due. The leads from the photocell were properly
insulated to avoid disturbances in the amplifier. The amplifier was of the
simplest type possible without elaborate controls and the details of its working

had already been fully described by Mr. R. Ananthakrishnan[4] in his paper on the " Photoelectric Photometry of Light Scattering in Liquids". It was found that by the use of good storage batteries, a steady state could be reached in the bridge within a short time after switching on the various connections.

The particular mixture under examination was maintained at the requisite temperature. The incident light was cut off by a chutter before it entered the mixture. The various connections in the amplifier circuit were switched on and the bridge was brought to a balance by adjusting the rheostat r_2 (see Fig. 1). The reading of the micro-ammeter was taken. The incident light was then let into the mixture and the final steady deflection of the micro-ammeter was again read off. The observations were repeated with the same mixture for a series of temperatures up to the critical solution temperature and also with the other mixtures. When the intensity of scattering was found to increase to large values as would be the case near the critical solution temperature, the intensity of the incident beam was proportionately reduced by means of the iris diaphragm attached to the long-focus lens so that the deflections of the micro-ammeter were always of the same order of magnitude.   •

The possible sources of error in the experiment are the following : (*a*) the want of linearity in the response of the amplifier, (*b*) the want of steadiness of the intensity of the light of the projection lantern which was used as the source of light, and (*c*) the absorption and reflections at the various surfaces. The linearity in the response of the amplifier was tested out by noting the deflections of the galvanometer using the same mixture as the scattering substance when the intensity of the incident light was reduced by known amounts. It was found that the deflections registered by the galvanometer in the bridge circuit when scattered light of a certain small intensity was allowed to fall on the photocell could be taken to be proportional to the intensity of light falling on the cell.

The intensity of light emerging out of the square aperture was measured in a foot-candle meter at regular intervals keeping the distance between the two standard carbon rods used in the projection lantern constant. It was found that the projection lantern could be taken as a fairly steady source of light within the limits of experimental error.

No account was taken of the absorption of light since the distances involved were small. In this case of the comparison of intensities, errors arising from the reflections at the various surfaces, etc., were automatically

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[4] R. Ananthakrishnan, *loc. cit.*

A2

eliminated by the use of bulbs of the same capacity for all the mixtures and by keeping the rest of the experimental arrangement undisturbed.

From the observed deflections of the micro-ammeter and from a knowledge of the proportions in which the intensity of the incident light was reduced in each case, the relative intensities of light scattered by the mixtures at various temperatures were calculated. The results are tabulated below.

TABLE I.

(a)   15% *Phenol.*

Temperature of the mixture °C.	P_h %	P_v %	P_u		Relative intensity of transversely scattered light
			(observed) %	(calculated) %	
88	100	1·5	3·1	3·0	1·0 (assumed)
78	100	1·1	2·3	2·2	1·3
69	100	0·75	1·4	1·5	2·0
61	100	0·55	1·1	1·1	3·8
60	100	0·5	1·0	1·0	8·3

TABLE II.

(b)   28% *Phenol.*

Temperature of the mixture °C.	P_h %	P_v %	P_u		Relative intensity of transversely scattered light
			(observed) %	(calculated) %	
90	100	0·9	1·7	1·8	1·0 (assumed)
87	84	0·7	1·5	1·5	1·2
81	70	0·5	1·0	1·2	1·7
77	59	0·34	0·84	0·9	2·4
74	49	0·27	0·76	0·82	3·9
72	35	0·23	0·7	0·88	5·8
68·5	22	0·15	0·62	0·83	23·8

TABLE III.

(c) 34% *Phenol.*

(*Critical Composition Mixture.*)

Temperature of the mixture °C.	ρ_h %	ρ_v %	ρ_u		Relative intensity of the transversely scattered light
			(observed) %	(calculated) %	
95	75	1·0	2·2	2·3	1·0 (assumed)
90	66	0·7	1·5	1·7	1·2
87·5	61	0·6	1·1	1·6	1·3
83	51	0·13	1·1	1·3	2·1
80	47	0·31	0·93	1·0	2·9
77	39	0·27	0·77	0·96	3·8
71	31	0·23	0·75	1·0	7·8
71	26	0·19	0·7	• 0·93	24·6
69·5	16	0·12	0·62	0·87	45·0
69	12	0·07	0·62	0·65	53·0

TABLE IV.

(d) 50% *Phenol.*

Temperature of the mixture °C.	ρ_h %	ρ_v %	ρ_u		Relative intensity of the transversely scattered light
			(observed) %	(calculated) %	
90	100	2·1	4·0	4·1	1·0 (assumed)
85	95	1·6	3·3	3·2	1·3
80	93	1·2	2·5	2·5	1·7
75	80	0·84	2·1	1·9	2·1
70	70	0·62	1·5	1·3	3·2
66·5	51	0·32	1·0	0·95	4·8
64·5	41	0·27	1·0	0·93	8·2

TABLE V.　(e)　60% *Phenol.*

Temperature of the mixture °C.	ρ_h %		(observed) %	(calculated) %	Relative intensity of the transversely scattered light
89	100	4·5	8·2	8·6	1·0 (assumed)
79	100	3·8	7·2	7·3	1·2
70	100	2·8	5·3	5·5	1·6
65	100	2·4	4·8	4·7	2·3
61	100	1·9	3·8	3·7	3·7

TABLE VI.　(f)　70% *Phenol.*

Temperature of the mixture °C.	ρ_h %	ρ_v %	ρ_u (observed) %	ρ_u (calculated) %	Relative intensity of the transversely scattered light
59	100	9·3	16·3	17·0	1·0 (assumed)
49	100	8·2	14·9	15·1	1·2
40	100	6·9	12·2	12·9	1·3
30	100	6·0	11·0	11·3	1·5

TABLE VII.

Composition of the mixture i.e., % by weight of Phenol	Temperature of miscibility °C.	ρ_h %	ρ_v %	ρ_u %	Relative intensity of opalescence
15	60	100	0·5	1·0	6·6
28	68·5	22	0·15	0·62	71
34	**69**	**12**	**0·07**	**0·62**	**186**
50	64·5	41	0·27	1·0	15
60	61	100	1·9	3·8	3
70	30	100	6·0	11·0	1 (assumed)

5. Discussion of Results.

In the tables given above the numbers given in the fifth column are the values of ρ_u calculated from the observed values of ρ_h and ρ_v using the reciprocity formula.[5] The observed values of ρ_u are in satisfactory agreement with the calculated values.

The experimental results show some very striking characteristics. For a mixture having any composition whatsoever, the values of the depolarisations ρ_u, ρ_v and ρ_h are minimum at the critical point, while the intensity of light scattered transversely is a maximum at this temperature. Again the depolarisations ρ_u, ρ_v and ρ_h attain minimum values and the intensity of scattering attains the maximum value for the mixture having the exact critical composition, *i.e.*, in this case for a mixture containing 34% by weight of phenol (see Table VII). The temperature of complete miscibility, as is well known, is also maximum for this concentration. As we go away from the critical composition on either side, the temperature of complete miscibility decreases, the depolarisation values increase suddenly at first and then gradually. In the same manner, the intensity of scattering falls off suddenly to less than half its value for the critical solution mixture, when the composition differs from the critical composition even by a small amount. For any mixture and especially for a critical composition mixture, there is a very rapid increase in the intensity of scattering as the critical solution temperature is approached. If curves are drawn showing (1) the dependence of depolarisation on composition, (2) the temperature of complete miscibility on composition, and (3) the intensity of scattering on composition, they will have approximately the same parabolic form.

It is seen from the results obtained that ρ_h for mixtures containing 15% by weight of phenol, 60% phenol and 70% phenol *has always the limiting value* of 100% showing thereby that no detectable clusters are present in these mixtures. In the case of the other three mixtures (*i.e.*, mixtures containing 28% phenol, 34% phenol and 50% phenol) detectable clusters are formed as is seen from the fall in the value of ρ_h from 100%. The range over which such clusters are detectable is less than 20° C. for mixtures containing 28% phenol and 50% phenol, while the range is more than 30° C. for a 34% phenol mixture. The values of ρ_h and ρ_v for the 34% phenol mixture at the critical solution temperature are considerably lower than the corresponding values for other mixtures. But the values of ρ_u is of the same order of magnitude. These definitely indicate that not only is the tendency for the formation of molecular clusters a maximum for the critical composition

5 R. S. Krishnan, *Proc. Ind. Acad. Sci.*, 1935, **1A**, 782.

mixture, but also that the size of the clusters formed is greatest for this mixture. These results are in accordance with the remark made by H. S. Taylor[6] in his book on Physical Chemistry, that *it is only with the critical composition in a system containing two partially miscible liquids that a critical state can be reached.*

6. *Relation to Other Physical Properties.*

Various other physical properties such as viscosity, flow birefringence, magnetic birefringence, etc., have been extensively studied by numerous investigators. Ostwald and Malss[7] have determined the coefficient of viscosity for a series of binary mixtures of the type phenol and water both above and below the critical solution temperature. Only mixtures having the exact critical composition have been studied by them. They find an abnormal increase in the viscosity at the critical point. They try to explain the same by saying that liquid mixtures at the critical solution temperature are emulsoid systems of approximately colloidal degree of dispersion. Ostwald and Erbring[8] further found a marked streaming double refraction for a mixture of carbon disulphide and methyl alcohol within a definite region of temperature in the neighbourhood of the critical solution temperature.

A. Piekara[9] has investigated the variation of magnetic birefringence with temperature at different concentrations in mixtures of nitrobenzene and normal hexane. He finds that the thermal coefficient of magnetic birefringence increases with concentration, the coefficient becoming abnormally large at the critical solution point. The results are explained by him by assuming that the increase in the molecular association causes an increase in the molecular and magneto-optic anisotropy. These abnormal properties exhibited by critical composition mixtures near the critical point are in all probability due to the formation of molecular clusters under these conditions.

Critical composition mixtures near the critical point cannot be regarded as colloidal solutions or emulsoids since some important characteristics of a colloidal solution are not exhibited by these mixtures. A colloidal system progressively changes with time. But it is a fact of observation that the critical opalescence and such other phenomena exhibited by critical solution mixtures are invariant with time and depend only on the temperature. Moreover, if liquid mixtures at the point of complete miscibility behave like emulsoid systems, then all the phenomena which are characteristic of

[6] H. S. Taylor, *A Treatise on Physical Chemistry*, Vol. 1, 530.
[7] W. Ostwald and A. Malss, *Koll. Zeits.*, 1933, **63**, 61.
[8] W. Ostwald and Erbring, *Koll. Zeits.*, 1933, **64**, 229.
[9] A. Piekara, *Jour. de Phys.*, 1934, **5**, 54.

the critical composition mixture should also be exhibited by mixtures whose compositions are widely different from the critical composition. But this is not so. Although large molecular clusters are formed in binary liquid mixtures at the critical solution temperature, there seems to be no justification for regarding them as colloidal systems.

In conclusion, the author wishes to record his indebtedness to Prof. Sir C. V. Raman, Kt., F.R.S., N.L., for his kind and helpful interest in the work.

7. *Summary.*

This paper deals with a comparative study of the intensity and depolarisation of the light scattered transversely by mixtures of phenol and water in different proportions and at different temperatures. The lowest values for the depolarisations ρ_u, ρ_v and ρ_h are obtained with the critical composition mixture (in this case 34% phenol mixture) at the critical solution temperature, where ρ_u, ρ_v and ρ_h are the depolarisations of the light scattered transversely when the incident light is (1) unpolarised, (2) polarised with electric vector vertical, and (3) polarised with electric vector horizontal respectively. The photoelectric method was employed for the comparison of intensities of scattering by these mixtures at various temperatures above the critical solution temperature. The intensity of scattering is found to increase considerably for the 34% phenol mixture as the critical solution temperature is approached. It is found that the tendency for the formation of clusters and the size of the clusters formed depend on the temperature and composition of the mixture. These phenomena are found to be most pronounced for the critical composition mixture at the critical solution temperature. The bearing of these results on the anomalies of viscosity, magnetic birefringence, etc., noticed in the vicinity of the critical conditions is also pointed out.

ON THE DIPOLE MOMENT OF TETRALIN.

By M. A. Govinda Rau, M.A., Ph.D.,

AND

S. Satyanarayana Rao, M.Sc.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

M. Puchalik[1] has recently reported that tetralin (tetrahydronaphthalene) exhibits in benzene solutions considerable association, and a dipole moment of value $1 \cdot 66 \times 10^{-18}$ e.s.u. Now, this value of moment is unusually high for a pure hydrocarbon. Experimental measurements have so far shown[2] that with the exception of the unsymmetrically substituted acetylene hydrocarbons, investigated by Otto and Wenzke,[3] almost the whole gamut of hydrocarbons of whatever shape and structure show moment values only between $0 \cdot 5$ to $0 \cdot 0$. Even here, the higher values are shown generally by unsymmetrically substituted ethylene compounds, and some benzene derivatives. More recently rather high values of moment have been reported by Svirbely, Albard and Warner[4] for *d*-pinene and *d*-limonene : these values, however, require confirmation, particularly as the measurements have been carried out at extremely dilute solutions where the experimental errors have enormous effect on the calculated values of moment.[5] It was, therefore, deemed necessary to check up the high value reported for the moment of tetralin and all the more so, as almost pure tetralin is available in commercial quantities and could be used as a solvent in dipole moment measurements.

Experimental.

The tetrahydronaphthalene used in these experiments was prepared from a "pure" stock, by drying over phosphorous pentoxide, and two successive slow fractional distillations in an all-glass apparatus under reduced pressure. The middle fraction boiling at a very steady temperature was collected for use : $d_4^{25°} = 0 \cdot 9639$, $n_D^{25°} = 1 \cdot 5382$.

The dielectric constant and density of the pure tetralin itself was measured initially, over the temperature range 10 to 40°, with the apparatus and methods described already.[6] The experimental values together with the

[1] M. Puchalik, *Acta. physic. Polon.*, 1933, **2**, 305–310. See *Sci. Abs.*, 1934, 1294.

[2] "Table of Dipole Moments, Class 2B," *Trans. Farad. Soc.*, 1934, 30.

[3] M. M. Otto and H. H. Wenzke, *J. Amer. Chem. Soc.*, 1934, **56**, 1314.

[4] W. J. Svirbely, J. E. Albard and J. C. Warner, *Ibid.*, 1935, **57**, 652.

[5] M. A. Govinda Rau and B. N. Narayanaswamy, *Zeit. phys. Chem.*, (B), 1934, **26**, 25.

[6] M. A. Govinda Rau and B. N. Narayanaswamy, *loc. cit.*

calculated values for the molecular polarisations are presented in Table I. It is at once evident from the low value for the dielectric constant, and the very slight *decrease* in molecular polarisation with temperature, that tetralin is a nearly non-polar liquid, whose molecules have a finite but small moment. For, experimental measurements on all definitely non-polar liquids have hitherto shown a small but finite *rise* in molecular polarisation values with temperature,[7] a behaviour which is satisfactorily accounted for on the basis of the Raman-Krishnan theory of liquid structure.[8] If it is assumed that the Debye equation can be directly applied to the polarisation values for the pure liquid state,[9] the calculated value for the moment of tetralin comes out as $0 \cdot 41 \times 10^{-18}$.

TABLE I.

Pure Tetralin.

Temperature in °C.	Dielectric constant (E)	Density (d)	$P \cdot \dfrac{E-1}{E+2} \cdot \dfrac{M}{d}$
10 ..	2·785	0·9760	50·51
20 ..	2·756	0·9679	50·42
30 ..	2·727	0·9599	50·30
40 ..	2·697	0·9519	50·16

The difficulties in determining to the usual degree of accuracy moments of this order of magnitude by the method of dilute solutions have been discussed frequently in the literature.[10] Thus, when the so-called "optical" method of calculating moment is employed, usually unsatisfactory results are obtained as there is no reliable method of determining the exact value of the induced or displacement polarisation. On the other hand when the method of temperature coefficient of molar polarisation is employed an increased accuracy, at least ten times in the dielectric constant and density values, is called for, or else the temperature range over which the molar polarisation is determined must be considerably enlarged. As in the present case benzene is

[7] C. P. Smyth and W. N. Stoops, *Jour. Amer. Chem. Soc.*, 1928, **50**, 1883; R. W. Dornte and C. P. Smyth, *Ibid.*, 1930, **52**, 3546; L. E. Sutton, R. G. A. New and J. B. Bentley, *Jour. Chem. Soc.*, 1933, 652; F. Fairbrother, *Proc. Roy. Soc.*, (A), 1933, **142**, 173; W. Lautsch, *Zeit. phys. Chem.*, (B), 1928, **1**, 115; H. Ulich, E. Hertel and W. Nespital, *Ibid.*, 1932, **17**, 369.

[8] C. V. Raman and K. S. Krishnan, *Proc. Roy. Soc.*, (A), 1927, **117**, 589; *Phil. Mag.*, 1928, **5**, 498; K. S. Krishnan, *Proc. Roy. Soc.*, (A), 1929, **126**, 155.

[9] L. M. Heil, *Phys. Rev.*, 1932, **39**, 666; J. Estermann, *Zeit. phys. Chem.*, (B), 1928, **1**, 134.

[10] See M. A. Govinda Rau and B. N. Narayanaswamy, *loc. cit.*

the solvent in which the high moment has to be examined, the temperature range over which measurements could be made stands limited. Further, as the methods and apparatus used to determine the dielectric constant and density were the same as before, it was hoped to obtain any improvement in accuracy by determining these values over a larger range of concentrations, particularly as with weakly polar solutes and non-polar solvents, the dielectric constant and density can be reasonably expected to be a linear function of concentration over a much larger range. This was realised in the present experiments. The slopes a and β of dielectric constant and density respectively with molar concentration were read off correct to $0 \cdot 001$ and $0 \cdot 0001$, and the $P_{2\infty}$ values calculated by the Hedestrand formula (Table II). The resulting slope of $P_{2\infty} - 1/T$ calculated by the method of least squares gave a moment value of $0 \cdot 40 \times 10^{-18}$ e.s.u.

This moment was also calculated by the "optical" method as a further check. There are here usually two methods employed, the one in which the total induced polarisation is taken as the molar refractivity for the sodium D line, and the other in which it is taken as equal to $MR_{\infty} + 15\%$ of same, where MR_{∞} is the extrapolated value of molar refractivity for infinite wave-length.

<div align="center">TABLE II.</div>

<div align="center">(i)</div>

Concentration in mol. fraction	10°		20°		30°		40°	
	E	d	E	d	E	d	E	d
0	2·303	0·8884	2·284	0·8777	2·265	·8669	·245	0·8562
0·01671	2·319	0·8908	2·300	0·8801	2·281	·8694	·263	0·8587
0·02750	2·329	0·8924	2·309	0·8818	2·289	·8712	·269	0·8606
0·03469	2·333	0·8930	2·314	0·8826	2·294	·8723	·274	0·8619
0·05201	2·350	0·8956	2·329	0·8852	2·309	·8748	·288	0·8644
0·06356	2·355	0·8970	2·334	0·8866	2·313	·8763	·292	0·8659
0·09105	2·378	0·9012	2·357	0·8909	2·337	·8808	·316	0·8706

The value of MR_D calculated from our data is $42 \cdot 91$ c.c.; and when subtracted from the value of $P_{2\infty}$ at $20°$, gives an orientation polarisation of $9 \cdot 51$ c.c., corresponding to a moment of $0 \cdot 67 \times 10^{-18}$. On the other hand

(ii)

$t°C.$	α	β	P_{200}
10 ..	0·820	0·1112	52·49
20 ..	0·803	0·1445	52·42
30 ..	0·789	0·1520	52·24
40 ..	0·781	0·1590	52·17

the value of MR_∞ calculated from the values of Auwers,[11] is $41·19$, and the moment calculated by the second method $0·49 \times 10^{-18}$. This value agrees better with those determined by the temperature coefficient methods. It is interesting that an exactly similar observation has been made in a recent paper by Briegleb and Kambeitz,[12] where it is found that a value of $1·15$ MR_∞ for the induced polarisation leads to a more passable value for the moment of symmetrical trinitrobenzene.

Discussion.

To a rather rough approximation tetralin could be regarded as an alkyl ortho disubstituted benzene derivative, and the moment should therefore be of the order of that of ortho-xylene, *i.e.*, of about $0·5 \times 10^{-18}$. This is just what has been observed for tetralin. The high value of moment reported by Puchalik is therefore improbable. A closer consideration of the structure of tetralin is at present difficult. While it is obvious that the two carbon atoms of the two CH_2 groups directly attached to the benzene ring must be in the plane of the ring, the other two carbon atoms in the remaining CH_2 groups can lie either in the plane of the other atoms or displaced from it. The structure of this saturated half of tetralin should be exactly analogous to that of cyclohexene (C_6H_{10}). A study of the moment of this compound should throw some light on the origin of the moment of tetralin, and the influence, if any, of the aromatic unsaturated half of tetralin on the aliphatic saturated half. Work on these lines is under progress.

Summary.

The moment of tetralin as measured in benzene as solvent is only $0·4$ to $0·5 \times 10^{-18}$, and not $1·66 \times 10^{-18}$ as reported by Puchalik. The origin of this small but finite moment is briefly discussed.

[11] V. Auwers, *Berichte*, 1913, **46**, 2990, quoted by Landolt Tabellen.

[12] G. Briegleb and J. Kambeitz, *Zeit. phys. Chem.*, (B), 1934, **27**, 11. See also G. Briegleb, *Zeit. phys. Chem.*, (B), 1932, **16**, 276.

DOPPLER EFFECT IN LIGHT SCATTERING IN LIQUIDS.

Part II. Polarisation of the Transversely Scattered Radiations.

BY B. V. RAGHAVENDRA RAO.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. *Introduction.*

IN previous communications, the author described experiments dealing with the examination of molecularly scattered light with the aid of a Fabry-Perot Interferometer and a specially-designed cathode-cooled low-density mercury vapour lamp.

In the first of these communications[1] which concerned itself with the study of the fine structure of the radiations scattered by liquid benzene in the backward direction, the author successfully demonstrated the reality of the two Doppler components appearing one on either side of the central undisplaced line. There was no indication, however, of the other components of higher frequency shifts reported by Gross.[2] The formula of Brillouin was also checked, the calculated values of the frequency shifts agreeing particularly well with the observed values for the mercury radiations, 4047 Å, 4078 Å and 4358 Å.

In the next paper[3] were reported some results concerning the relative intensities of the central undisplaced line to the outer Doppler components in the two liquids, carbon tetrachloride and toluene, chosen specially in view of the fact that the first liquid has optically isotropic molecules, while the latter has a relatively high degree of molecular anisotropy. The central undisplaced component appeared with great intensity in carbon tetrachloride, which indicates that the optical anisotropy of the molecules is not primarily responsible for the appearance of this central component. The Fabry-Perot pattern with toluene showed a strong continuous spectrum overlapping the triplet. This feature was almost entirely absent in the pattern obtained with carbon tetrachloride. From this it may be concluded that the continuous

[1] B. V. Raghavendra Rao, *Proc. Ind. Acad. Sci.*, 1934, **1**, 261.

[2] E. Gross, *Nature*, 1930, **126**, 400.

[3] B. V. Raghavendra Rao, *Proc. Ind. Acad. Sci.*, 1935, **1**, 473.

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spectrum is a Raman effect due to the hindered rotation or angular oscillation of the molecules in the liquid, the intensity of this being naturally a function of the optical anisotropy of the molecules.

The third paper of the series[1] dealt with the influence of change of temperature of the liquid on the fine structure of the scattered radiation. On heating liquid carbon tetrachloride to 70° C., the Doppler components on either side of the central undisplaced component became fainter and broader, and tended to merge with the central line which brightened up in intensity. The Doppler components were also found to approach the central line with rise of temperature. These results suggest that the Brillouin theory of the Doppler effect departs from the truth, as the temperature of the liquid is raised.

The present paper describes a study of the state of polarisation of the three components observed in the spectrum of the scattered light when examined with a Fabry-Perot etalon. Beyond a casual statement by Gross[5] in one of his communications to *Nature*, very little work appears to have been done on this subject. Hence it was thought important to undertake a careful examination of this question. For this purpose, it is necessary to observe the light scattered in the transverse direction, though this direction is not so well suited for a separation of the Doppler components from the central line, owing to their closer approach to it.

2. *Experimental Arrangements.*

Since in this case we have to examine the transversely scattered light, a horizontal type of low-density cathode-cooled mercury vapour lamp has been designed as shown in Fig. 1. The intensity of this lamp is enhanced
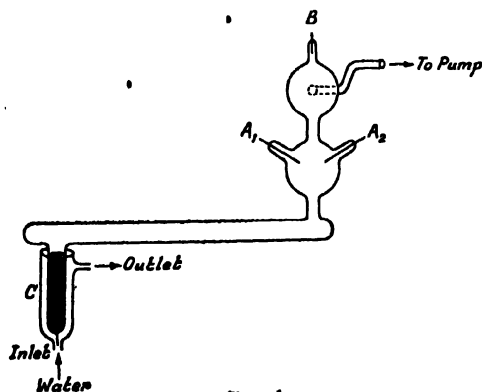
FIG. 1.

4 B. V. Raghavendra Rao, *Proc. Ind. Acad. Sci.*, 1935, **1**, 765.

5 E. Gross, *Nature*, 1930, **126**, 400.

by running two arcs simultaneously with the aid of the two anodes and the common mercury cathode. A parallel beam of light from this lamp is made to illuminate the side of the Wood's tube along its axis, by means of a big 8″ condenser. A large square-ended Nicol is placed between the observation window of the Wood's tube and the Fabry-Perot etalon, and the emerging interference pattern of the scattered light is focussed on to the slit of a Füess spectrograph by a lens. Two separate spectra are recorded in each case, one with the Nicol having its vibration axis vertical and the other with the vibration axis horizontal. Three liquids have been studied, namely, carbon tetrachloride, toluene and carbon disulphide, chosen as representative cases of molecules having respectively no optical anisotropy, moderate anisotropy and a high degree of anisotropy.

3. Results.

Carbon tetrachloride.—In the vertical position of the vibration axis of the Nicol, the interference pattern of the scattered light is characterised by the appearance of the central line and the two Doppler components on a very clear background. In the horizontal position of the Nicol, nothing was recorded on the plate, in spite of extended exposures of the order of 48 hours. The results indicate that all the three components are completely polarised, and that a continuous spectrum if it is present is extremely weak.

Toluene.—In the vertical position of the vibration axis of the Nicol, all the three components are present and can be observed in the interference pattern of the scattered light superimposed on a continuous background. In the horizontal position of the Nicol, only the continuous background is recorded in the spectrum, and no trace of the three components could be detected with certainty.

Carbon disulphide.— The results are very similar to those in the case of toluene except that the continuous spectrum is somewhat stronger. Exposures ranging from a few hours to 48 hours were given with the vibration axis of the Nicol horizontal, to see if the presence of any of the three components could be detected superimposed on the continuous background. There were very faint indications of the presence of Fabry-Perot rings crossing the continuous background, and it was uncertain as to whether they belonged to the central undisplaced line or the Doppler components. It may be mentioned here that any experimental defects such as the angle of scattering being indefinite by reason of the use of a source of finite length, as also the presence of any parasitic light, ought to have, if anything, given a central undisplaced line in the horizontal component of the scattered light.

The plate reproduces the interference patterns of the three liquids as negatives for clearness of reproduction.

4. Significance of the Results.

So far as the two Doppler components are concerned, we should expect them to be completely polarised, as is indicated by the observations, since they arise from density fluctuations due to the presence of sound waves of various wave-lengths associated with the thermal energy of the medium. The further result indicated by observation, namely, that the central undisplaced component is also completely polarised seems at first sight very surprising, especially when we remember that the polarisation of transversely scattered light is very imperfect to the extent of 46% in toluene and 62% in the case of carbon disulphide. We know however that the central undisplaced line appears with great intensity also in the case of carbon tetrachloride, and its appearance is therefore not primarily ascribable to the optical anisotropy of the molecule. This makes it easier to understand why it should be more or less completely polarised. We may assume that the undisplaced line is due to the scattering of light by individual molecules or by groups of molecules in the liquid which are free to rotate or oscillate. The Q branch in the spectrum of the scattered light due to such molecules or groups of molecules should be more or less completely polarised. For instance, in the case of carbon disulphide, the total scattering by individual molecules would show a defect of polarisation only to the extent of 11% and if we consider only the Q branch the defect would be only a fraction of this, of the order of a few per cent. The Q branch in the scattering by groups of molecules, unless they are highly unsymmetrical in shape, should be even more completely polarised.

These observations and the foregoing remarks make it clear why in the horizontal component of the scattering by liquids, the Q branch, if present at all, is extremely weak. Further, it follows that practically the whole of the depolarisation of the light scattered by liquids at ordinary temperatures is to be ascribed to the presence of a continuous spectrum of radiation of altered wave-length. The presence of such continuous radiation in the light scattered by liquids and its marked imperfection of polarisation were discovered simultaneously with the presence of sharp lines of altered wave-lengths.[6] The spectral character of this radiation, namely, that of a nebulosity or wings accompanying the primary and the displaced lines in the spectrum of the scattered light is shown by the very earliest photographs and microphotometer records of Raman spectra.[7] The fact that this continuous

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[6] C. V. Raman, *Ind. Jour. Phys.*, 1928, 2, 395 ; *Nature*, 1928, 121, 619.

[7] C. V. Raman, *Nature*, 1928, 121, 711.

C. V. Raman and K. S. Krishnan, *Ind. Jour. Phys.*, 1928, 2, Plate 13, Fig. 6 and Plate 16.

spectrum is almost completely unpolarised, that its intensity increases with the optical anisotropy of the molecule and that it is unsymmetrical about the lines which it accompanies, led Raman and Krishnan[8] to suggest that it arises from the impeded rotations of the molecules in a dense fluid. Though much has been written on the subject, this original explanation still holds the field if we add the qualification that the impeded rotation may take the form of approximately periodic angular oscillations of the molecule and that account is also taken of the impeded rotation or oscillation of groups of molecules.[9]

In view of what has been said above, it is difficult to attach much significance to the measurements which have been published of the depolarisation of light scattering in liquids made spectroscopically with slits of varying width. Mr. S. Venkateswaran[10] who made a series of measurements of this kind showed that the depolarisation of light scattering diminishes as the slit width employed is narrowed. He also remarked that this diminution is much less conspicuous for highly associated liquids such as acetic acid, formic acid,...,etc., than for non-associated liquids, e.g., benzene and carbon disulphide. Rousset[11] who has pursued the subject further has shown that by using still finer slits, values for the depolarisation lower than those reported by Venkateswaran may be obtained. If we are to credit the results obtained in the present paper with the Fabry-Perot etalon, the depolarisation thus measured is simply a function of the resolving power of the instrument, and still lower values should be obtained by using spectrographs of great power and the finest slits possible. This is a subject which appears well-worth pursuing, in order to confirm the provisional conclusion that the Q branch is practically absent in the horizontal component of the scattering by liquids, and to ascertain the size of the molecular groups present and responsible for the continuous spectrum given by the various types of liquids. It appears desirable also to extend the work with the Fabry-Perot etalon using highly associated liquids such as formic and acetic acids, and also to re-examine the case of non-associated liquids using fine slits and an auxiliary spectrograph of greater power as to eliminate the obliterating effect of the superimposed continuous spectrum. Quantitative estimates of the imperfection of polarisation of the central undisplaced component would evidently be desirable in all these cases. Work on these lines is in progress.

[8] C. V. Raman and K. S. Krishnan, *Nature*, 1928, **122**, 278 and 882; *Proc. Roy. Soc.*, 1929, **122**, 30.

[9] S. Bhagavantam, *Proc. Ind. Acad. Sci.*, 1935, **2**, 63.

[10] S. Venkateswaran, *Phil. Mag.*, 1932, **14**, 258.

[11] Rousset, *C. R.*, 1933, **197**, 1033.

Carbon tetrachloride
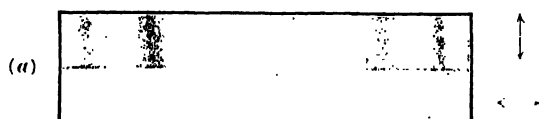
(*a*)

Toluene

(*b*)

Carbon disulphide

(*c*)

In conclusion the author desires to express his grateful thanks to his professor Sir C. V. Raman, for his kind interest and helpful guidance during the progress of this work.

Summary.

The present paper describes a study of the state of polarisation of the three components observed in the spectrum of the scattered light when examined with a Fabry-Perot etalon. Three typical liquids, carbon tetrachloride, toluene and carbon disulphide have been examined. The results indicate that the two Doppler components are completely polarised as is to be expected, and that the central component is practically completely polarised. The significance of the latter result which appears surprising at first sight, is discussed in some detail.

A3

A THEOREM ON THE ADDITION OF RESIDUE CLASSES : APPLICATION TO THE NUMBER $\Gamma(k)$ IN WARING'S PROBLEM.

By INDER CHOWLA.

1.   Davenport[1] has recently proved the following remarkable Theorem :

Let p *be a prime ; let* $a_1, \cdots, a_m$ *be* m *different residue classes* (mod p) ; *let* $\beta_1, \cdots, \beta_n$ *be* n *different residue classes* (mod p). *Let* $\gamma_1, \cdots, \gamma_l$ *be all those different residue classes which are representable as*

$$a_i + \beta_j \quad (1 \leqslant i \leqslant m, \ 1 \leqslant j \leqslant n).$$

Then $1 \geqslant m + n - 1$ *provided* $m + n - 1 \leqslant p$, *and otherwise* $1 = p$.

In this paper we generalize this theorem to any composite modulus. We can only do this by introducing certain restrictions on one of the sequences.   In fact it is easy to show by an example that the theorem is not, as it stands, true for non-prime moduli.

Example.   Let the modulus be 81, and let the a's be the positive integers $\equiv 1 (9)$ and less than 81, so that $m = 9$; let the β's be the positive integers $\equiv 2(9)$ and less than 81, so that $n = 9$.   Then it is easy to see that the γ's are the positive integers $\equiv 3(9)$ and less than 81, so that $l = 9$.   Hence $l = m + n - 9$ so that the theorem is not true.

Our theorem, which is not a complete generalization, is as follows :

Theorem 1.   Let k *be a positive integer ; let* $a_1, \cdots, a_m$ *be* m *different residue classes* (mod k) ; *let* $\beta_1, \cdots, \beta_n$ *be* n *different residue classes* (mod k) *where one of the* β's *is* 0 *and the rest are prime to* k.   *Let* $\gamma_1, \cdots, \gamma_l$ *be all the different residue classes which are representable as*

$$a_i + \beta_j \quad (1 \leqslant i \leqslant m, \ 1 \leqslant j \leqslant n).$$

Then $1 \geqslant m + n - 1$ *if* $m + n - 1 \leqslant k$, *and otherwise* $1 = k$.

That the number $m + n - 1$ in this theorem cannot be replaced by a greater number is shown by the following

Example.   Let the a's be the positive integers $\equiv 1(9)$ and less than 81 ; let the β's be the number 0 and the positive integers $\equiv 2(9)$ and less than 81

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[1] *Journ. London Math. Soc.*, 1935, **10**, 30-32.

81. Then $m=9$, $n=10$. The γ sequence consists of the positive integers $\equiv 1(9)$ and $\equiv 3(9)$ and less than 81. Hence $l=18$, and $l=m+n-1$.

2. This theorem has applications to the number $\Gamma(k)$ in Waring's problem. Let us define $\Gamma(k)$ as the least s such that every arithmetical progression contains an infinity of numbers of the form $x_1^k + \cdots + x_s^k$ where the x's are integers. Hardy and Littlewood[2] have proved that $\Gamma(k) \leqslant 4k$, while $\Gamma(k) = 4k$ for infinitely many k. It is an easy consequence of theorem 1 that

Theorem 2. Let $s \geqslant 4k$, and let the integers a_r ($r \leqslant s$) be prime to a. Then, every arithmetical progression $ax + m$ contains infinitely many numbers of the form

$$a_1 x_1^k + \cdots + a_s x_s^k$$

where the x's are integers.

The particular case $a_1 = \cdots = a_s = 1$ gives $\Gamma(k) \leqslant 4k$. Detailed proofs will appear elsewhere.

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[2] "Some Problems of ' Partitio Numerorum': IV," *Math. Ztschr.*, 1922, **12**, 161-188 (Theorem 12, page 186).

MAGNETIC DOUBLE REFRACTION AND LIGHT SCATTERING IN FUSED NITRATES.

By V. N. THATTE,

Department of Physics, College of Science, Nagpur.

The paper gives an account of measurements on the light scattering and magnetic double refraction of some fused nitrates. The magnetic and optical anisotropies of the NO_3 group are calculated therefrom, and are found to be the same as for the NO_3 group, in nitric acid and in crystalline nitrates.

1. *Introduction.*

IN a recent investigation on the Raman spectra of fused inorganic nitrates[1] it was observed that the Raman lines characteristic of the nitrate ion appear in practically the same positions as in the spectra of aqueous solutions of the nitrates, suggesting that the structure of the ion is the same in both the states. In order to find whether other physical properties of the ion, as for example its magnetic and optical anisotropies, are also the same in fused nitrates as for the free ion in aqueous solution, we have made some experimental studies on light scattering and magnetic double refraction of fused nitrates. The present paper gives a report on these studies. Fused zinc and cadmium nitrates were chosen for these studies, since they could be easily maintained in super-cooled condition over a wide range of temperatures.

2. *Light Scattering.*

(i) *Polarisation of the scattered light.*—The depolarisation factor of the scattered light from the fused nitrates was measured by the usual method of Cornu with a double image prism and a nicol. It is not necessary to give here the experimental details since they are so well known. The following table gives the values of the ratio, r, of the weak component to the strong one in the transversely scattered light, when the incident light is unpolarised. The values of the depolarisation factor of scattered light from nitric acid of different concentrations are given below for comparison.

(ii) *Intensity of the scattered light.*—The intensity of the light scattering of the fused nitrates was measured by comparing it with the scattering by benzene at room temperature. This liquid was chosen for the comparison

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[1] Thatte and Ganesan, *Ind. Jour. Phys.*, 1934, **8**, 341.

[2] S. Venkateswaran, *Ind. Jour. Phys.*, 1926, **1**, 235.

TABLE I.

Temp. °C.	Depolarisation factor, r	
	Zinc nitrate	Cadmium nitrate
30	·51	·44
46	·50	·43
65	·48	·41
81	·45	..
128	·36	·33
140	·31	·30

Strength of acid %	14·1	28·0	39·2	16·0	64·7
r	·36	·11	·18	·57	·61

since its absolute scattering has been measured accurately by Cabannes and others.

The experimental method adopted was as follows: A strong narrow beam of parallel light was allowed to through a cell with plane glass windows, containing dust-free benzene. The transmitted beam was then allowed to pass through a similar vessel containing the fused nitrate. The tracks of light in the two liquids were of nearly the same cross-section, and their intensities were compared with the help of an Abney rotating sector. The cell containing the fused nitrate was then replaced by a similar cell containing dust-free benzene, and again the intensity was compared with that of the benzene in the first cell. From these two measurements the intensity of the light scattering by the fused nitrates relative to that of benzene is known. (This method naturally eliminates the effects of any deviation from parallelism of the incident light.)

The measured intensities were as below. Taking the transverse scattering by benzene at 30° C. as unity, the scattering of fused zinc nitrate at 65° C. was 1·18, and that of fused cadmium nitrate at 70° C. was 1·40.

3. Magnetic Double Refraction.

The fused nitrate was kept in a double-walled tube, and was heated by a steady stream of hot water from a thermostat, circulating between the two walls ; the temperature was maintained constant to within a degree.

The magnetic double refraction was measured by the arrangement described in detail by Chinchalkar in a recent publication.[3] The double refraction was compared with that of pure acetone, with the help of a Rayleigh strained glass compensator.

The experimental results are given in the following table. C_m denotes the double refraction, defined as usual by the expression

$$C_m = \frac{n_{\parallel} - n_{\perp}}{\lambda H^2} \qquad \cdots \qquad \cdots \qquad \cdots \qquad \cdots \qquad (1)$$

The second column in the table gives the values of C_m measured relatively to that of acetone, and the third column gives the absolute values calculated on the assumption that for acetone

$$C_m = \cdot016 \text{ times that of nitrobenzene}$$
$$= \cdot4 \times 10^{-13}, \text{ for } \lambda = 589 \ m\mu.$$

Zinc nitrate	1·4	$5 \cdot 6 \times 10^{-14}$
Cadmium nitrate	1·25	$5 \cdot 0 \times 10^{-14}$

4. The Optical Anisotropy of the NO_3 ion.

From the scattering data given in the previous section, the optical anisotropy of the NO_3 group can be calculated. In the first place it is known from X-ray measurements on the crystalline nitrates that the NO_3 group has a plane structure, with the three O atoms at the corners of an equilateral triangle and the N atom at the centre. With such a structure the two optical polarisabilities of NO_3 group in its plane should be equal and much greater than that along the normal to the plane. Let $A = B$ and C be respectively the three polarisabilities. Let us assume that in the fused state the nitrate is more or less completely ionised, i.e., that the metallic ions, viz., Zn^{++} or Cd^{++} are not permanently attached to NO'_3. Since these metallic ions are isotropic, the depolarisation of the scattering will be determined practically by the optical anisotropy of the NO_3 group, the metallic ions influencing it only indirectly through their contribution to the refractive index, and to the compressibility of the medium.

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[3] Ind. Jour. Phys., 1932, 7, 491.

Defining as usual the optical anisotropy δ of the NO'_3 ion by the equation

$$\delta = \left(\frac{A-C}{2A+C}\right)^2 \qquad .. \qquad .. \qquad .. \qquad .. \qquad (2)$$

the calculation of the anisotropy is easily made, since the necessary expressions have been derived by the earlier workers. We have

$$r = \frac{6\delta}{5 \, kT\beta \, \nu + 7\delta} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (3)$$

where ν is the number of NO'_3 ions per c.c., k is the Boltzmann constant, T is the absolute temperature, and β is the compressibility. All the quantities in the above expression are known, except β, which can, however, be obtained indirectly from the intensity of the scattered light.

Calculating in this manner we find

$$\beta = 101 \times 10^{-6} \text{ per atm. for zinc nitrate}$$

and 121×10^{-6} per atm. for cadmium nitrate.

Substituting the values of the various quantities in (2), we obtain

$$\delta = \cdot 038 \text{ for the ions in zinc nitrate}$$

and $\cdot 036$ for the ions in cadmium nitrate,

from which we obtain

$$\frac{A}{C} = 1 \cdot 9.$$

Thus the optical polarisability along directions in the plane of the NO_3 group is about twice that along the normal to the plane.

5. *Comparison with Values of the Anisotropy obtained from Other Data.*

The above value for the ratio $\frac{A}{C}$ agrees well with the value $\frac{A}{C} = 2 \cdot 0$ obtained from the depolarisation of light scattering by nitric acids of different concentrations.[4]

It is also possible, following the method of Bragg,[5] to calculate the anisotropy of the NO'_3 ion from the birefringence of crystalline sodium nitrate, in which the NO'_3 ions are all oriented parallel to one another and perpendicular to the trigonal axis. The ratio of the refractivity of the NO_3 ions in the crystal for light vibrations normal to the trigonal axis to that for vibrations along the axis is found to be $1 \cdot 67$. In order to obtain $\frac{A}{C}$ for the *free* NO_3 ion from the above ratio, we have to correct for the influence

[4] Krishnan and Raman, *Proc. Roy. Soc.* (A), 1927, 115, 549.

[5] W. L. Bragg, *Proc. Roy. Soc.* (A), 1924, 106, 346.

of the surrounding sodium and NO'$_3$ ions in the crystal. Making this correction we obtain

$$\frac{\Lambda}{C}=1\cdot 8$$

which is nearly the same as the value obtained in the previous section.

6. Magnetic Anisotropy of the NO$_3$ ion.

The Cotton-Mouton constant C_m is given by the expression

$$C_m = \frac{(n^2-1)\,(n^2+2)\,(A'-C')\,\sqrt{\delta}}{30\,n\lambda\,kT} \qquad \cdot\cdot \qquad \cdot\cdot \qquad \cdot\cdot \quad (4)$$

Since the value of the optical anisotropy of the NO$_3$ group is now known, the above relation can be utilized to calculate the magnetic anisotropy of the ion, viz., $(A'-C')$ from the known value of the Cotton-Mouton constant. We thus obtain

$$\cdot A'-C'=4\cdot 4\times 10^{-6} \text{ per gram ion for zinc nitrate}$$

and $4\cdot 0\times 10^{-6}$ per gram ion for cadmium nitrate.

These values are of the same order as that obtained by Krishnan and Raman from the magnetic double refraction of nitric acid, viz., $5\cdot 3\times 10^{-6}$ per gram ion for nitric acid.

In sodium and potassium nitrates it is well known from X-ray data that the NO$_3$ groups are arranged in parallel planes so that the difference between the principal susceptibilities of the crystals for directions in the plane of the NO$_3$ group and along the normal to their planes should be equal to $A'-C'$. The magnetic anisotropy[6] for these crystals is equal to $4\cdot 9\times 10^{-6}$ per gram ion.

Thus we find that both the optical and the magnetic anisotropies of the NO$_3$' ion in fused crystals are practically the same as for these ions in other states, viz., in state of solution and in crystals.

The author wishes to record his heart-felt thanks to Prof. K. S. Krishnan for his advice and keen interest in the work.

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[6] Krishnan, Guha and Banerjee, *Phil. Trans.* (A), 1933, 231, 235.

DIAMAGNETISM OF COPPER.

By Dr. S. Ramachandra Rao,

Annamalai University.

1. *Introduction.*

In a recent paper,[1] the author has reviewed at some length the present position of our knowledge regarding the magnetic properties of colloidal particles of metals. A few general conclusions emerge from our perusal of the literature on this subject. Substances like graphite (which is not a metal) and bismuth (which is not a metal in the true sense of the term) show a decrease in the diamagnetic susceptibility value on colloidalisation. The elegant experiments of Krishnan and Ganguli[2] with blue graphite not merely confirm the effect in the case of graphite but also indicate definitely that the direction of largest decrease of susceptibility on colloidalisation is the one parallel to the hexagonal axis. To explain the high diamagnetism of these elements, it was suggested by Ehrenfest[3] and Raman[4] that some of the valency electrons had large orbits. Such orbits could not be possible on the surface and hence increase of surface area by colloidalisation would bring about a decrease in the specific diamagnetic susceptibility. This explanation obviously implies that the particles are much less densely packed on the surface than in the interior. The effect therefore of a smaller density of distribution on the surface is to decrease the effective specific diamagnetic susceptibility of the specimen and in the case of colloidal particles, the surface area is sufficiently large to decrease effectively the mean value of the diamagnetic susceptibility of the powder.

In the case of good conductors like copper, conditions are quite different. The valency electrons are considered here as free, the number of such electrons being of the same order as the number of atoms in the metal. Honda and Shimizu[5] have considered this question at some length and have quantitatively accounted for the increase of diamagnetism on cold-working.

Due to the expansion on cold-working, there is a diminution of free electrons and hence a decrease in the paramagnetic component due to them. According to Pauli,[6] this decrease $\delta \chi_1$ is given by

$$\delta\chi_1 = \frac{2}{3} \frac{C.L^{1/3}}{W^{1/3}} \left(-\frac{2}{3} \rho^{-5/3} a^{1/3} \delta\rho + \frac{1}{3} a^{-2/3} \rho^{-2/3} \delta a \right) \quad .. \quad (1)$$

where $C = 2 \cdot 21 \times 10^{-14}$, L = the Loschmidt number, W = the atomic weight, ρ = the density, and a = the number of free electrons per atom.

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At the same time there should be an increase in the diamagnetic component due to the increase in the number of bound electrons, since on expansion some of the otherwise free electrons get associated with atoms. Sommerfeld[7] has determined this increase $\delta\chi_2$ as

$$\delta\chi_2 = \frac{3 \cdot 1 \times 1 \cdot 84 \times 10^{-5}}{W} \cdot \frac{1}{3} a^{-2/3} \delta a \qquad \qquad .. \qquad .. \quad (2)$$

where $\delta a = \frac{A}{3}\left(\frac{4\pi\rho}{3M}\right)^{0.488} \frac{\delta\rho}{\rho}$

in which $A = 2 \cdot 261 \times 10^{-12} Z^{0.513}$ and M = mass of the atom, as calculated by Honda, Nishina and Hirone.[8] The total change in the susceptibility is given by $\delta\chi = \delta\chi_1 + \delta\chi_2$.

The net effect of cold-working is consequently to increase the diamagnetic susceptibility and this is exactly the result of the observations of Honda and Shimizu.

They have more recently[9] drawn attention to the close relations between colloidalisation and cold-working in metals. They explain that in the case of tin, the lattice constant is a little larger in the surface layer than in the interior, the normal value for the metal being reached at some hunderd layers below the surface. Thus colloidalisation should bring about increased diamagnetism quite similar to what is obtained in the case of cold-working. This was exactly the observation made by the writer.

The obvious interest aroused by this investigation led the author to undertake an inquiry into the behaviour of copper. Copper is of particular interest since it is a typical metal being a good conductor of electricity. Honda and Shimizu have investigated the effect of cold-working on this element and shown that there is an increased diamagnetism if the necessary corrections are made for the presence of ferromagnetic impurities. Copper crystallises in the cubic system with a face-centred structure, the side of the elementary cube being 3·597 A.U.[10] Any directional variations are thus out of question.

2. *Experiment.*

(a) *Purity of the specimen.* — The specimens of copper used were in the form of rods 5 mm. diameter, obtained from Adam Hilger. They were the purest available and had been subjected to a thorough chemical and spectroscopic examination. Hilger's report gave the following impurities in the specimen.

Iron	.. ..	0·0041%
Nickel	.. ..	0·0004%
Tin ..	.. ..	0·0005%
Lead	.. ..	0·0004%
Bismuth	.. ..	Trace.

Thallium	..	..	Trace.
Calcium	..	..	$0 \cdot 0005\%$
Magnesium	..	..	$0 \cdot 0001\%$
Zinc	..	..	Trace.
Oxygen	..	..	$0 \cdot 030\%$
Total Impurities		..	$0 \cdot 036\%$

The amount of copper present in the specimen was thus $99 \cdot 964\%$ which should be regarded as more than sufficient for our purposes. The question of the presence of ferromagnetic impurities will be considered later.
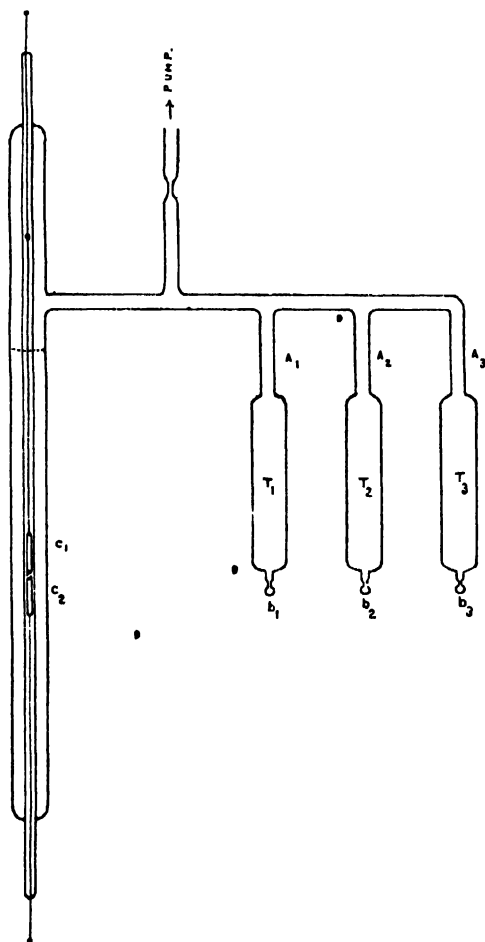
FIG. 1.

(b) *Preparation of the colloidal powders.*—The apparatus[11] used for colloidalisation is shown in Fig. 1. Two lengths of copper c_1 and c_2 were

cut to be about 2 cms. each and attached to two stout copper leads, such that the free ends faced each other. A gap of about 1 mm. was left as the sparking distance between the ends. After thoroughly cleaning the tube, pumping out, warming and drying, the sparking tube was filled with normal propyl alcohol or benzene. The tube was connected once again to the pump and after bringing down the pressure to the lowest obtainable, sparking was allowed to take place by connecting the leads to the secondary of an induction coil. The sparking currents were kept low, 2 to 8 milliamperes to minimize the heat produced ; and the time of sparking ranged from $\frac{1}{2}$ to about $1\frac{1}{2}$ hours.

The coarser particles were then allowed to settle for about an hour and the pump connection was now sealed off. The clear liquid was then allowed to flow in T_1 till it was nearly full and the tube was next sealed off at the constriction drawn at A_1. Thus the liquid was allowed to fill successively the tubes T_2 and T_3, each tube being sealed off after filling.

The particles in these tubes were next allowed to settle by gravitation or were centrifuged. They were then filed a bit at the top and were broken open in vacuum. The liquid was drained off and by placing the entire tube in a heater, the remaining liquid was evaporated. The small bulbs were then sealed off.

After observing the magnetic deflections, the bulbs were broken open and the size of the particles determined with a high power microscope having an eye-piece scale which was calibrated by observing the lines of a diffraction transmission grating. The particles were mixed with a little propyl alcohol as otherwise they appeared in clusters in the field of view.

The glass bulbs were cleaned thoroughly with warm dilute nitric acid and then with hot distilled water and dried The magnetic deflections were next observed for the bulbs alone.   •

In all cases the bulbs were weighed before and after removing the copper particles. The masses of the powders varied from about 10 to 40 mg., the error in weighing being less than $0\cdot1$ mg.

(c) *Measurements of the susceptibility.*—The Curie method was adopted to determine the specific susceptibilities of powders. Full details of this method and the calculation of results are given in an earlier paper.[12]

3. *Results.*

(a) *Mass metal.*—Small chips of copper were cut and pickled thoroughly with hydrochloric acid. They were then washed and dried. Several samples were investigated and the results for a few specimens at infinite field strengths are given below.

TABLE I.

Sample No.	Mass in grams	Diamagnetic susceptibility χ
1	0·2235	0·0805
2	0·1872	0·0803
3	0·2478	0·0798
4	0·1690	0·0800
5	0·2020	0·0799
6	0·1993	0·0797
7	0·2101	0·0798

The results give for copper in bulk a mean specific diamagnetic susceptibility of 0·080. This value compares favourably with those of other investigators given below.

TABLE II.

Investigator	Diamagnetic susceptibility χ
M. Owen[13]	0·085
K. Honda[14]	0·086
St. Meyer[15]	0·071
Koenigsberger[16]	0·088
Honda and Shimizu[17]	0·0818
Author[18]	0·080

The susceptibility of the specimens was determined at different field strengths. According to Honda,[19]

$$\chi_i = \chi_p + \frac{\sigma m}{H}.$$

where χ_p is the specific susceptibility of the pure sample, χ_i the corresponding value for the impure specimen and σ and m the specific intensity of magnetisation and mass of the ferromagnetic impurity present in unit mass of the

specimen. A graph drawn between χ_i and $\frac{1}{H}$ gives a straight line which could be extrapolated and the susceptibility determined for $\frac{1}{H} = 0$ or $H = \infty$. In this manner the disturbing effect of the ferromagnetic impurity can be eliminated.

In Fig. 2 are plotted the results obtained for four specimens. The diamagnetic susceptibility in all the cases works to a value in the immediate neighbourhood of 0·080.
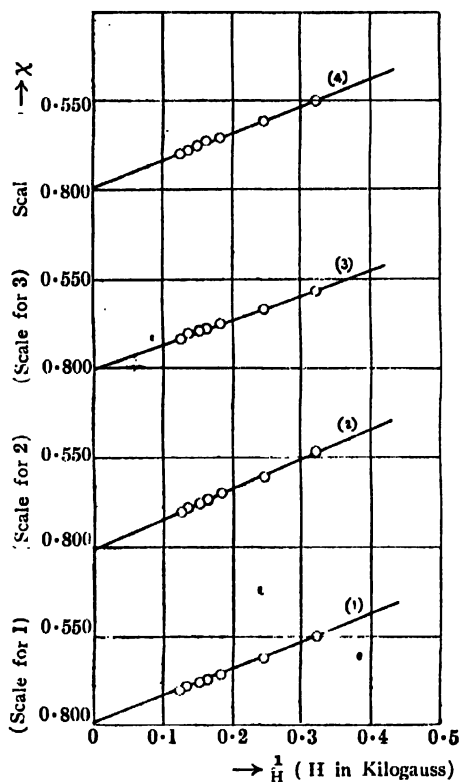
FIG. 2.

(b) *Colloidal particles.*—The results obtained for a dozen samples are tabulated below. For each sample, measurements were made at different field strengths and the value at infinite field strengths was obtained by extrapolating the χ, $\frac{1}{H}$ graph. The diameters of the particles in any sample were the same to within 5%. Fig. 3 shows the graph between the diameter of the particles and the susceptibility.
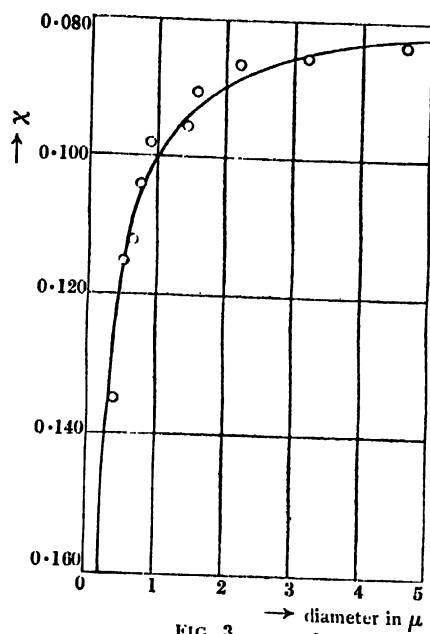
FIG. 3.

TABLE III.

Diameter of the particles	Mass in mg.	Dispersing medium	χ
10·0	38·8	b	0·083
6·0	40·2	b	0·083
3·2	32·1	p	0·085
2·2	23·7	b	0·086
1·6	26·3	p	0·090
1·5	27·2	b	0·095
1·0	30·2	b	0·094
0·9	18·6	p	0·098
0·75	17·7	p	0·100
0·65	21·3	b	0·112
0·5	17·4	p	0·115
0·4	19·5	b	0·135

b—Benzene ; p—Propyl alcohol.

The susceptibility increases slightly as the particle diameter decreases to $0.8\,\mu$. The value increases more rapidly at smaller particle sizes attaining 0.135 at the smallest diameter obtained in this investigation, $0.4\,\mu$. The critical diameter below which a large increase in the susceptibility occurs is $0.8\,\mu$. This is much smaller than the corresponding values for bismuth[20] ($1.4\,\mu$), graphite[20] ($1.5\,\mu$) and tin[21] ($2.0\,\mu$).

It will also be noticed that the diamagnetic susceptibility depends only on the dimensions of the particles and not on the nature of the dispersing medium—benzene or propyl alcohol.

Fig. 4 shows the X, $\frac{1}{H}$ curves for a few of the powders indicated. A point of great importance emerges from a scrutiny of the graphs. The
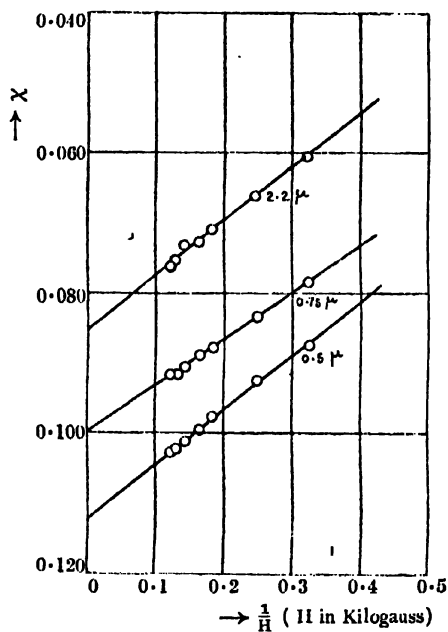
FIG. 4.

slopes of the straight lines are nearly the same, indicating that the ferromagnetic impurity occurs in the same state in all the powders investigated or that colloidalisation is not accompanied by any separation of the iron content causing spurious effects to enter into the magnetic measurements.

(c) *Heating the powders.*—A full description of the heating arrangement has been given in an earlier paper. Bits of copper and a few of the colloidal samples were heated and at each temperature for any specimen, the susceptibility at different field strengths was determined. It was found that the

diamagnetic susceptibility extrapolated to infinite field strengths was the same for any specimen at temperatures ranging from 30° C. to 350° C. Heating did not also change the slope of the χ, ι curves, indicating that the nature of the iron content was the same at all temperatures investigated.

4. Discussion.

We shall first consider the possibility of impurities giving rise to increased diamagnetism on colloidalisation. A reference[22] to the values of the susceptibilities of copper compounds indicates that the ordinary compounds of copper are either feebly paramagnetic or less diamagnetic than the metal. Further it has already been pointed out that the susceptibility of the colloidal powders depends only on the dimensions of the particles and not on the nature of the liquid in which colloidalisation is effected. It is thus most probable that the increase of diamagnetism with decreasing particle size is a genuine effect. This observation receives confirmation from the experiments of Honda and Shimizu on the effect of cold-working on the susceptibility of copper.

It was mentioned in section 1 that these authors observed a small increase of diamagnetism when copper was subjected to cold-working. The specific diamagnetic susceptibility changed from 0·848 to 0·926 (an increase of 0·078) as the density of the metal diminished from 8·9521 to 8·6646. On the basis of equations (1), (2) and (3), the calculated value of $\delta\chi$ for this change of density works to 0·061, which agrees very well with the observed value.

Honda and Shimizu have suggested that in the case of colloidal powders, the lattice constant is a little greater in the surface layer than in the interior, the normal value for the metal being reached at some hundred layers below the surface. Thus colloidalisation should be accompanied by increased diamagnetism quite similar to what is obtained in the case of cold-working. This is in accord with experiment.

The results with colloidal powders do not seem to lend themselves to a straight calculation of the susceptibility of the surface layer and its thickness. However by trial, it was found that the results fitted closely with the calculated values assuming the thickness of the surface layer to be $0 \cdot 03\,\mu$ and its diamagnetic susceptibility 0·200. In fact in Fig. 3, the continuous line has been drawn to give the results calculated on the basis of these assumptions and it is found that the points plot themselves near the line.

The surface layer thus seems to be about 300 A.U. thick and hence contains nearly 80 atomic layers.

It follows from (1), (2) and (3) that

$$\delta\chi = K\,\delta\rho$$

where K is a constant for any given metal, *i.e.*, that the change in the susceptibility is directly proportional to the change in density. On calculation, it is found that $K = -0.2226 \times 10^{-6}$ for copper. If the surface layer has a specific diamagnetic susceptibility of 0.200, then $\delta X = 0.120$ which gives for $\delta\rho$ a value of 0.539. Since the density of the mass copper was found to be 8.913, that of the surface layer should be 8.404. It would be of interest to check this result by direct experiment but no density measurements seem to have been obtained so far with colloidal powders.

There is however one experimental fact which lends support to the view that the density of the superficial layer is less than the density of the mass metal. It is obvious that the lattice constant should be larger at the surface than in the interior. To investigate the surface layers, the recent electron diffraction experiments have come in very handy. G. P. Thomson[23] studied the surfaces of metallic single crystals by the electron reflection method. In these experiments only a few atomic layers are concerned. He deposited copper electrolytically on the etched surface of a copper crystal and discovered by electron diffraction methods that the spacing of part at least of the deposit was larger than that of normal copper, though the structure was roughly similar. The deposit was probably in the form of very minute crystals on the copper base and these gave rise to the new spacing presumably because of their smaller density. In fact the spacing in certain cases was found to be 4.5 A.U. while that of ordinary copper is 3.597 A.U.

5. Summary.

Colloidal copper was prepared by an electrical dispersion method, the dispersing medium being benzene or propyl alcohol. The particles were prevented from coming into contact with air by conducting the dispersing and centrifuging experiment *in vacuo*. The diamagnetic susceptibility (X) of the samples was determined by a sensitive Curie method. The value for copper *en masse* was found to be 0.080. The X value increased as the size of the particles was reduced, the critical diameter below which large changes occurred being 0.8μ.

Honda and Shimizu have shown that cold-working in the case of copper gives rise to increased diamagnetism. They have pointed out that due to the expansion on cold-working of the metal, there is a decrease in the paramagnetic component due to the diminution of free electrons and an increase in the diamagnetic component due to the increased number of bound electrons. They explain that the lattice constant is a little greater in the surface layer than in the interior and suggest the analogy between cold-working and colloidalisation. These conclusions are verified in the case of copper. The

thickness of the surface layer appears to be about 300 A. U. and its diamagnetic susceptibility 0·200. On the basis of the theory of Honda and Shimizu, we obtain for the density of the superficial layer a value of 8·404 as against 8·943 for the mass metal. Attention is drawn to the experimental work of Thomson on electron diffraction from electrolytic copper, wherein larger lattice constants were obtained for surface layers.

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THE RAMAN SPECTRUM OF PHOSPHORUS.

By C. S. VENKATESWARAN.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. *Introduction.*

BHAGAVANTAM[1] investigated the Raman effect with yellow phosphorus in the solid state using the green and yellow radiations of the mercury arc as the exciting lines. He gives the characteristic frequencies as 607 (6), 468 (3) and 374 (1); the figures in the brackets being the intensities as recorded with the 5160 Å. U. excitation. He has discussed his results theoretically,[2] considering the two alternate forms for the tetra-atomic molecule, namely, that of a square and of a tetrahedron respectively. The former model would give four different frequencies of oscillation, while the tetrahedron would give three frequencies. Accordingly, the experimental results are regarded as supporting the tetrahedral model for P_4 which is in agreement with the fact that the yellow phosphorus crystallises in the cubic system.[3] For the particular law of forces adopted, the frequencies should have the ratio $2 : \sqrt{2} : 1$; the fact that the observed frequencies deviate from this ratio is regarded as indicating that the force system in the molecule is more complicated than that assumed. In the present paper the Raman spectrum of yellow phosphorus has been critically studied with the substance in four different states: (1) vapour, (2) liquid, (3) solid and (4) solution in carbon disulphide. Such a comparative study is evidently of interest in order to ascertain the influence of the state of aggregation on the characteristic frequencies of the molecules, and has so far been made in the case of very few substances. In the present investigation, some additional bands have been recorded besides those reported by Bhagavantam and a small shift has been observed in the frequency of the most intense line as we pass from the solid to the liquid and to vapour.

2. *Experimental Arrangements and Results.*

The experimental arrangements were the same as those described in the previous communications of the author[4] in these *Proceedings.* Small

[1] Bhagavantam, S., *Ind. Jour. Phys.,* 1930, **5**, 35.

[2] Bhagavantam, S., *Ind. Jour. Phys.,* 1930, **5**, 73.

[3] Natta and Passerini, *Nature,* 1930, **125**, 707.

[4] Venkateswaran, C. S., *Proc. Ind. Acad. Sci.,* 1935, **1**, 850.

transparent pieces of yellow phosphorus were kept immersed in distilled
water and exposed in the vertical tube, the solid being kept at the room
temperature by the circulation of cold water in an outer jacket surrounding
the experimental tube. Liquid phosphorus was formed by the heating of
the yellow crystals contained in an evacuated tube, by the heat of the mercury
arc itself. The solution in carbon disulphide was prepared by dissolving
clean pieces of yellow phosphorus in freshly distilled carbon disulphide con-
tained in a closed tube during exposure. The arrangement for the vapour
is shown in Fig. 1. A few bits of phosphorus were contained in a thick-
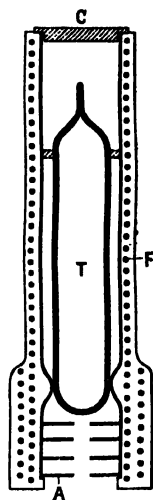
FIG. 1.   T = Tube, F = Furnace, A = Aperture, C = Cap.

walled pyrex tube, which was evacuated, rendered moisture free, and sealed
off. The tube was supported vertically as illustrated, inside another tube
over which nichrome wire was wound in order to serve as an electric furnace.
A few additional turns of heating coil at the lower end of the furnace prevented
the condensation of the vapour in the bottom of the tube. Once the phos-
phorus was vaporised, it was found necessary to keep the temperature high
enough in order to prevent the formation of red phosphorus. With a
current of about 4·5 amperes in the heating coil, the temperature inside the
tube remained almost steady at 450° C. A series of apertures were used
on the observation side so that the reflection from the walls of the tube was
completely eliminated.

The time of exposure was four hours for the solid, twelve hours for the
liquid and the solution and six days for the vapour at a pressure of about
15 atmospheres. The spectrograms were measured with a Hilger Cross-Slide

Micrometer in comparison with an iron arc spectrum which was recorded in the centre of every plate.

TABLE I.

Solid Phosphorus (Temperature 30° C.).

Exciting line	Raman lines		$\Delta\nu$ in cm.$^{-1}$	Intensity
	λ	ν		
5790·5 (ν = 17265)	6000·0	16662	603	3
,,	5950·6	16800	465	1
5769·6 (ν = 17328)	5977·5	16725	603	3
,,	5928·3	16864	464	1
5460·7 (ν = 18308)	5646·0	17706	602*	8
,,	5602·2	17845	463	4
,,	5573·3	17938	370(?)	0
4358·3 (ν = 22938)	4476·0	22335	603	2
,,	4447·9	22177	461	1

* This line is followed by an unresolved wing.

TABLE II.

Liquid Phosphorus (Temperature 75° C.).

Exciting line	Raman lines		$\Delta\nu$ in cm.$^{-1}$	Intensity
	λ	ν		
5790·5	6001·5	16659	606	6
,,	5951·6	16800	465	3
5769·6	5979·0	16720	606	6
,,	5928·6	16863	465	3
5460·7	5647·4	17702	606	8
,,	5602·5	17844	464	4

TABLE III.

Phosphorus Vapour (Temperature 450° C.).

Exciting line	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity
	λ	ν		
5460·7	5649·5	17696	612	3
,,	5603·0	17813	465 (diffuse)	1

TABLE IV.

Solution in CS_2 (52%).

Exciting line	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity
	λ	ν		
5460·7	5661	17650,	658	3
,,	5619	17697	611	8
,,	5603	17813	465	3

In addition to the lines tabulated above, a strong fluorescent band has also been recorded in all the four plates with its centre at 6400 Å. U. and extending from 6230 Å. U. to 6550 Å. U., which is evidently due to the phosphorus itself. The strong line at 602 in the solid is accompanied by a faint wing to the red side, which would probably resolve into a companion line at higher dispersions. The faint line at 374 reported by Bhagavantam[5] has appeared only as a faint darkening on the plates of the solid and the liquid and the author is, hence, unable to confirm the reality of that line.

The influence of change of state on the Raman lines is not shown to any marked extent. The line at 602 in the solid is shifted slightly towards a higher frequency while passing from the solid to the liquid and hence to the vapour, the variation in frequency being of the order of four wave-numbers from the solid to the liquid and six wave-numbers from the liquid to the vapour. The shift remains almost the same in the two states of the vapour and the solution. The line at 461 appears unshifted in all the four states;

[5] Bhagavantam, S., *Loc. cit.*

but in the case of vapour it has become slightly more diffuse. Only small variations in the frequency have been observed in the past in the case of the non-polar compounds like H_2 or O_2,[6] while the polar molecules like hydrochloric acid[7] give in general large shifts. In the case of sulphur, the author[8] has not been able to observe any change in the frequencies from the solid to the molten conditions. Hence we are led to conclude that the small shifts observed in phosphorus while passing from the solid to the vapour are only such as could be accounted for by the Lorentz-Lorentz forces and shows that the P_4 molecule is non-polar. It may also be of interest to mention that though the frequency shifts in the vapour and solution are almost identical, the spectrum of carbon disulphide itself is comparatively feeble.

In conclusion the author wishes to thank Prof. Sir C. V. Raman for his kind interest in the work.

Summary.

The Raman spectrum of yellow phosphorus as vapour, liquid, solid, and solution in carbon disulphide has been obtained. Only small changes in frequency are observed while passing from solid to liquid and to vapour, indicating the non-polar character of the molecule. The spectrum of carbon disulphide is rendered weak although the spectrum of phosphorus in solution comes out strongly. A strong fluorescent band extending from 6230 Å. U. to 6550 Å.U. has also been recorded in all cases.

[6] Kohlrausch, *Der Smekal-Raman Effect*, 1931, p. 126.
[7] Callihan and Salant, *Jour. Chem. Phys.*, 1934, **2**, 317.
[8] Venkateswaran, C. S., *Proc. Ind. Acad. Sci.*, 1934, **1**, 120.

THE KINETICS OF HETEROGENEOUS ORGANIC REACTIONS: THE REACTION BETWEEN BENZYL CHLORIDE AND SOLID SILVER NITRATE.

BY M. V. NABAR

AND

T. S. WHEELER.

(From the Department of Chemistry, Royal Institute of Science, Bombay.)

Introduction.

IN connection with a systematic study which is being made in these laboratories on the kinetics of heterogenous organic reactions[1] we have investigated the reaction between solid silver nitrate and benzyl chloride in the absence of all solvents and diluents. No previous work of this type appears to have been carried out on the reaction. Burke and Donnan[2] (1904), Donnan and Potts[3] (1910) and Senter[4] (1910) investigated the reaction between silver nitrate and alkyl iodides in alcoholic and aqueous alcoholic solutions and have found the reaction to be complicated by the presence of the solvent.

Materials Used.

Silver Nitrate.—Experiments were carried out with Kahlbaum's "purest for analysis", Merck's "purest guaranteed reagent", and Johnson's "double crystallized" silver nitrate. It was found that all these different samples gave results agreeing within the experimental error. To obtain reproducible results, it was essential to have the particles of silver nitrate of average uniform size. This was accomplished in the following way :—

Silver nitrate was ground in a pestle and mortar in dim diffused light, and then sieved through a silk cloth of fine mesh to remove small particles, and then through a slightly bigger mesh. What passed through the second mesh was collected. The particles were examined under a microscope and the sieving repeated until they were of an average uniform size, and gave a standard rate of reaction under standard conditions. The standard reaction for medium size particles was :—

1 *J. Phys. Chem.*, 1935, **39**, 727, 901, 907.
2 *J. C. S.*, 1904, **85**, 555.
3 *J. C. S.*, 1910, **97**, 1882.
4 *J. C. S.*, 1910, **97**, 346.

Temperature	Benzyl chloride	Time of reaction	Initial silver nitrate	Silver nitrate reacting
35°C. ± 1°	5 c.c.	10 mts.	2 gm.	1·85 ± ·05

The sieving cloths and the process of sieving were standardized before beginning the set of experiments.

Three different sizes of particles denoted "Small", "Medium" and "Large" were employed. No difficulty was experienced in preparing fresh samples of the sizes.

It was observed that the presence of moisture considerably inhibits the reaction (see Fig. 4). Hence the samples of silver nitrate were kept in the

	Small size S	Medium size M	Large size L
Mesh of fine sieve ..	L 0·019 cm.	L 0·028 cm.	L 0·048 cm.
	B 0·017 cm.	B 0·024 cm.	B 0·037 cm.
Mesh of coarse sieve ·	L 0·023 cm.	L 0·031 cm.	L 0·057 cm.
	B 0·017 cm.	B 0·026 cm.	B 0·049 cm.
Average weight of a particle ..	$1·216 \times 10^{-5}$ gm.	$4·272 \times 10^{-5}$ gm.	$4·145 \times 10^{-4}$ gm
Number of particles per gm. ..	82402	23407	2412
Average volume of a particle ..	$2·795 \times 10^{-6}$ c.c.	$9·82 \times 10^{-6}$ c.c.	$9·53 \times 10^{-5}$ c.c.
Average dimensions of a particle ..	L 0·031 cm.	L 0·048 cm.	L 0·093 cm.
	B 0·019 cm.	B 0·03 cm.	B 0·055 cm.
	T 0·0047 cm.	T 0·00682 cm.	T 0·0186 cm.
Average surface area of a particle ..	0·00165 sq. cm.	0·00394 sq. cm.	0·0158 sq. cm.
Surface area of one gm. ..	136 sq. cm.	92·2 sq. cm.	38 sq. cm.

dark, in a desiccator over phosphorus pentoxide. At intervals the reaction velocities of these samples were checked in standard experiments.

The sizes of the meshes of different cloths, and the sizes and surface area of particles are given in the above table.

The average surface area of a particle was determined as follows:—

A fraction of a gram of the particles was weighed and the number of particles was counted. From this, the average weight of a particle and the number of particles per gram were calculated. Then about fifty particles were examined under a microscope. The shape of all these three types was observed to be cuboid. The length and breadth of each particle was measured under the microscope by means of a standard scale placed in the eye-piece. From this the average length I, and the average breadth B of each particle were calculated. From the average weight of each particle, and the density of silver nitrate (4·35), the volume of each particle was determined. Hence from the knowledge of the volume and I, and B, the average thickness T of each particle was calculated. The surface area was calculated from the formula 2(L × B + I, × T + B × T).

Benzyl Chloride.—Kahlbaum's benzyl chloride " purest " was used throughout the set of experiments. Its properties were not altered by fractionation under reduced pressure. The standard rate of reaction of a sample of benzyl chloride decreases on keeping for some weeks. The effect is marked with smaller amounts (less than one gram) of silver nitrate, where the impurity in the benzyl chloride is relatively more important. The decrease is probably due to the hygroscopic nature of benzyl chloride. Accordingly, it was preserved in small lots in amber-coloured bottles under anhydrous conditions.

Experimental Methods.

Benzyl chloride was measured by means of a burette, protected from moisture, into a small conical flask. It was confirmed that Jena-glass bottles gave the same result. A thermometer was fixed through the cork, and the flask was covered with black paper and was kept in an air thermostat adjusted to 35° ± ·2°C.

When the bottle attained the required initial temperature, sieved silver nitrate previously weighed in a weighing bottle and kept in a desiccator was added and the stop-watch started. The bottle was shaken by hand to mix the reactants thoroughly—this shaking is necessary—and it was then placed on the shaker which was also fixed in the thermostat, and the latter was started. The speed of rotation of the shaker was kept constant by means of a rheostat in the circuit of the motor. It was confirmed that variation of

speed of shaking between 75 and 180 revolutions per minute did not affect the rate of reaction (*see* Tables IV and V).

After a definite interval of time, the bottle was removed from the shaker, the temperature was read and the silver salts were rapidly filtered through a gooch crucible and washed free from benzyl chloride with dry ether or acetone. The receiver was then changed and the residual silver nitrate was washed down with distilled water. This washing was titrated with standard ammonium thiocyanate. In this way the amount of silver nitrate unused was obtained. This *minus* the initial amount gave the silver nitrate used.

In some experiments the results were checked by gravimetric estimation of the residual silver chloride. The volumetric and gravimetric results agreed excellently.

TABLE I.

Small Size Particles.

$AgNO_3$ initial	Benzyl chloride	Time	Temp. initial	Temp. final	$AgNO_3$ left	$AgNO_3$ used	K
gm.	c.c.	mts.	°C.	°C.	gm.	gm.	
1	5	3	34	35	0·387	0·613	0·0020
1	5	5	34	35·3	0·18	0·82	0·0019
1	5	7	34	35·5	0·033	0·967	0·0021
1	20	3	34	35	0·404	0·596	0·0019
1	20	5	34	35	0·194	0·806	0·0019
1	20	7	34	35·3	0·072	0·928	0·0018
3	5	3	33	37·5	0·73	2·27	0·0027
3	5	5	33	35·5	0·30	2·70	0·0025
3	5	7	33	37	0·04	2·96	0·0024
3	20	3	34	36	1·065	1·935	0·0021
3	20	5	34	36	0·27	2·73	0·0024
3	20	7	34	35·5	0·065	2·935	0·0023

Control of Reaction Temperature.

The reaction is exothermic and with the larger amounts of silver nitrate the rise in temperature is marked. As the heat of combustion of benzyl nitrate has not been determined, the heat change in the reaction cannot be calculated.

After a number of experiments it was found that by starting a little below 35° C. and by adjusting a wet cloth of suitable dimensions around or at the bottom of the bottle it was usually possible to keep the average temperature of the reaction close to 35° C. The degree of the wetness of the cloth and the extent to which the bottle was covered with it for different systems was determined in preliminary experiments. It was confirmed that the reaction is not sensitive to small temperature variations.

Experimental Results and Discussion.

TABLE II.

Medium Size Particles.

AgNO$_3$ initial	Benzyl chloride	Time	Temp. initial	Temp. final	AgNO$_3$ left	AgNO$_3$ used	K
gm.	c.c.	mts.	°C.	°C.	gm.	gm.	
1	5	2·5	35	35·7	0·60	0·10	0·0020
1	5	5	35	34	0·32	0·68	0·0020
1	5	7·5	35	37	0·164	0·836	0·0020
1	5	10	35	36	0·07	0·93	0·0019
1	10	2·5	35	36·6	0·62	0·38	0·0019
1	10	5	35	34·5	0·37	0·63	0·0018
1	10	7·5	35	35	0·19	0·81	0·0018
1	10	10	35	35·3	0·07	0·93	0·0019
1	20	2·5	35	35	0·65	0·35	0·0017
1	20	5	35	35·6	0·352	0·648	0·0019
1	20	7·5	35	35·6	0·144	0·856	0·0020
1	20	10	35	37	0·07	0·93	0·0019
2	5	2·5	34	37	1·19	0·81	0·0020
2	5	5	34	37	0·66	1·34	0·0020

TABLE II—(*Contd.*)
Medium Size Particles.

AgNO$_3$ initial	Benzyl chloride	Time	Temp. initial	Temp. final	AgNO$_3$ left	AgNO$_3$ used	K
gm.	c.c.	mts.	°C.	°C.	gm.	gm.	
2	5	7·5	34	36·6	0·29	1·71	0·0020
2	5	10	34	38	0·10	1·90	0·0020
2	10	2·5	34	35	1·30	0·70	0·0017
2	10	5	34	36·5	0·70	1·30	0·0019
2	10	7·5	34	36	0·28	1·72	0·0021
2	10	10	34	36	0·20	1·80	0·0017
2	20	2·5	34	35·4	1·25	0·75	0·0019
2	20	5	34	36	0·70	1·30	0·0019
2	20	7·5	34	35·5	0·36	1·64	0·0019
2	20	10	34	35·3	0·20	1·80	0·0017
3	5	2·5	33	36	1·70	1·30	0·0022
3	5	5	33	36·5	0·80	2·20	0·0023
3	5	7·5	33	36·5	0·40	2·60	0·0021
3	5	10	33	37	0·26	2·74	0·0018
3	10	2·5	33	35·3	1·70	1·30	0·0022
3	10	5	33	35·5	0·86	2·14	0·0022
3	10	7·5	33	36·3	0·40	2·60	0·0021
3	10	10	33	37·5	0·25	2·75	0·0018
3	20	2·5	34	36	1·80	1·20	0·0020
3	20	5	34	36	1·00	2·00	0·0020
3	20	7·5	34	38	0·40	2·60	0·0021
3	20	10	34	37	0·25	2·75	0·0018

TABLE III.

Large Size Particles.

AgNO$_3$ initial	Benzyl chloride	Time	Temp. initial	Temp. final	AgNO$_3$ left	AgNO$_3$ used	K
gm.	c.c.	mts.	°C.	°C.	gm.	gm.	
1	5	5	34	35	0·678	0·322	0·0020
1	5	15	34	36	0·24	0·76	0·0020
1	5	25	34	35·7	0·096	0·904	0·0017
1	20	5	35	35	0·678	0·322	0·0020
1	20	15	35	33·5	0·274	0·726	0·0018
1	20	20	35	34	0·15	0·85	0·0019
1	20	25	35	35·2	0·096	0·904	0·0017
3	5	5	34	35	2·08	0·92	0·0018
3	5	15	34	35·5	0·678	2·322	0·0021
3	5	20	34	35	0·46	2·54	0·0018
3	5	25	34	34	0·323	2·677	0·0016
3	20	5	34	33·6	2·10	0·90	0·0018
3	20	15	34	35·5	0·654	2·346	0·0021
3	20	25	34	33·5	0·186	2·814	0·0019

Tables I, II, III, and Figs. 1, 2 and 3 give the results of the experiments. It will be seen that the rate of the reaction is independent of the initial amount of benzyl chloride. That is to say, if we take two grams of silver nitrate, it matters not as far as the rate of reaction is concerned whether we start with 5 c.c., 10 c.c. or 20 c.c. of benzyl chloride. This is a striking result, since with smaller amounts of benzyl chloride, the concentration of benzyl nitrate in the liquid reaction mixture increases rapidly. One would expect therefore the rate of reaction to fall off more quickly than with larger initial quantities of benzyl chloride.

We can explain this result by assuming that benzyl chloride is adsorbed on the surface of the silver nitrate crystals, and that the amount of adsorption does not depend greatly on the concentration of benzyl chloride in the

mixture. There is therefore always a constant concentration of benzyl chloride on the silver nitrate surface, and the rate of reaction is consequently proportional to the surface of silver nitrate present, so that during an experiment the interface between silver nitrate and silver chloride will travel inwards through the crystal at a constant linear rate.
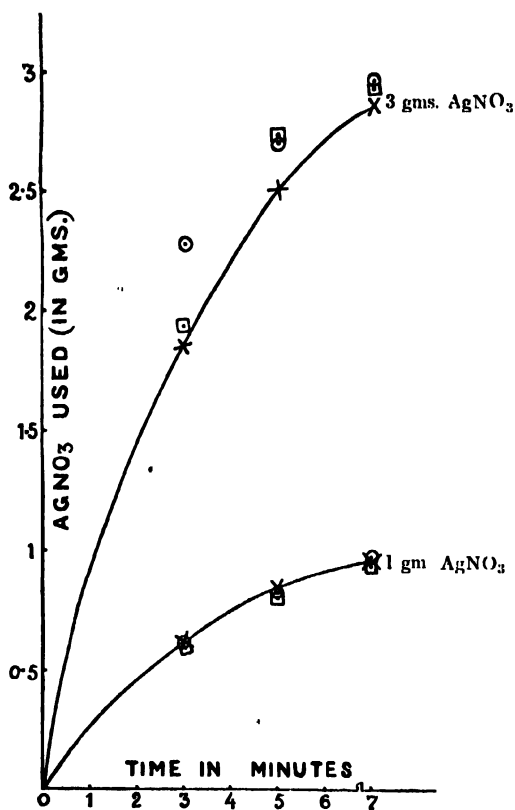
FIG. 1.   Small Size AgNO₃ Particles.

Benzyl Chloride + AgNO₃

⊙   5 c.c.   + 1 & 3 gms.
▱   20 c.c.   +   ,,
×   Calculated from K = 0·002

This type of reaction has been found by Spencer and Topley[5] (1929), to apply to the decomposition of silver carbonate. They point out that an equation of the type deduced below applies strictly only to particles of uniform size, but is approximately true as an average result when factors such

[5] *J. C. S.*, 1929, 2633.
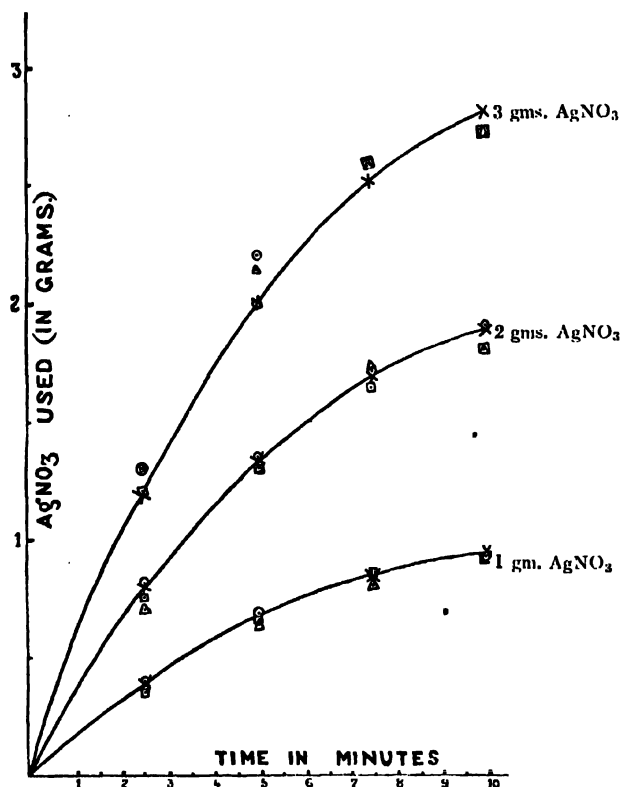
FIG. 2.   Medium Size $AgNO_3$ Particles.

Benzyl Chloride + $AgNO_3$

⊙	5 c.c.	+ 1, 2 & 3 gms.
△	• 10 c.c.	+ ,,
▣	20 c.c.	+ ,,
✕	Calculated from K = 0·002	

as irregular shape and different reaction rates parallel to different crystal axis are averaged over the large number of particles contained in the reactive material.   They also point out that reactants can be assumed to pass to and from the crystal interface by means of micro-fissures in the crystals.

With substances with large molecules such as benzyl chloride and benzyl nitrate this hypothesis is not so probable.   On the other hand the silver chloride formed around the silver nitrate particles may have a loose porous form, that is to say, a continuous crystal lattice is not formed but rather an agglomeration of particles.

A5   F
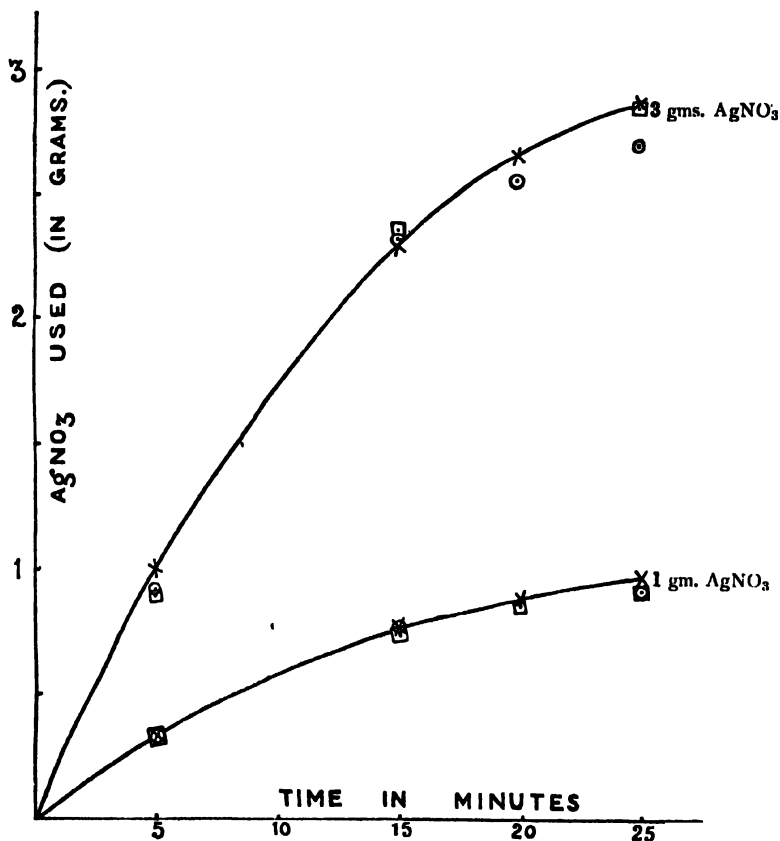
FIG. 3. Large Size AgNO₃ Particles.

Benzyl Chloride + AgNO₃

⊙   5 c.c. | 1 & 3 gms.
▣   20 c.c.   +   ,,
✗ Calculated from K = 0·002

It must also be emphasised that while in many liquid phase heterogeneous reactions, the chemical effect is obscured by conditions of physical transport, yet in many other instances the chemical effect predominates. Roller[6] (1935) cites a number of reactions in which diffusion does not play a part, e.g., Schmidt and Durau[7] (1924) showed that the rate of solution of glass in alkali is independent of the rate of stirring, and the concentration of alkali, but depends on the surface of the glass exposed. In our experiments the con-

[6] J. Phys. Chem., 1935, 39, 221.
[7] Z. Phys. Chem., 1924, 108, 128.

centration of benzyl chloride in the reacting medium is always high being never less than 60% by weight. This may tend to minimise diffusion effects, since there are always a large number of molecules near the reacting surface.

The real test of the hypothesis, however, is to examine how far the corresponding kinetic equation can reproduce the experimental results.

We deduce the kinetic equation as follows :—

Let A be the intial amount of silver nitrate in gms. and let X be the amount left at a time t. Let K be the number of gms. of silver nitrate reacting per sq. cm. of silver nitrate surface per second. Let $\bar{S}$ be the surface in sq. cms. of one gm. of the silver nitrate initially used.

Now if the silver nitrate particles be taken on an average to be spheres of the calculated surface area, the surface of the X gm. remaining after time t will be equal to

$$\frac{(\bar{S}A)\,X^{\frac{2}{3}}}{A^{\frac{2}{3}}} = \bar{S}\,A^{\frac{1}{3}}\,X^{\frac{2}{3}}$$

Hence

$$-\frac{dX}{dt} = K\bar{S}A^{\frac{1}{3}}X^{\frac{2}{3}}\dots\dots\dots\dots\dots\dots\dots\dots(1)$$

$$-dt = \frac{dX}{K\bar{S}A^{\frac{1}{3}}X^{\frac{2}{3}}}\dots\dots\dots\dots\dots\dots\dots\dots(2)$$

$$t = \frac{3A^{\frac{1}{3}}}{K\bar{S}A^{\frac{1}{3}}}\left(1 - \frac{X^{\frac{1}{3}}}{A^{\frac{1}{3}}}\right)\dots\dots\dots\dots\dots,\dots(3)$$

$$K = \frac{3}{tS}\left(1 - \frac{X^{\frac{1}{3}}}{A^{\frac{1}{3}}}\right)\dots\dots\dots\dots\dots\dots(4)$$

The values of K calculated from (4) are shown in Tables I, II and III and the constancy of the values indicates the general correctness of the assumed mechanism. A more stringent test is to use (4) to calculate the experimental results.

Examination of all experimental results indicate that K has an average value of $0\cdot002$. Hence as the small size (S) of silver nitrate used has a surface area per gm. of 136 sq. cm. we have for this sample the kinetic equation

$$0\cdot002 = \frac{3}{t(136)}\left(1 - \frac{X^{\frac{1}{3}}}{A^{\frac{1}{3}}}\right)\dots\dots\dots\dots\dots(5)$$

For the medium size (M) with the surface area per gm. of $92 \cdot 2$ sq. cm. we have

$$0 \cdot 002 = \frac{3}{t(92 \cdot 2)} \left(1 - \frac{X^{\frac{1}{3}}}{A^{\frac{1}{3}}} \right) \quad \dots \dots \dots \dots \dots \dots (6)$$

And for the large size (L) with the surface area per gm. of 38 sq. cm.

$$0 \cdot 002 = \frac{3}{t(38)} \left(1 - \frac{X^{\frac{1}{3}}}{A^{\frac{1}{3}}} \right) \quad \dots \dots \dots \dots \dots \dots (7)$$

From (5), (6) and (7), X has been calculated for various values of t, and the results are shown by full lines in Figs. 1, 2 and 3.

It will be seen that the equation reproduces well the experimental results. The agreement is all the more striking when it is remembered that for all these three results involving three different sizes of silver nitrate only one constant $K = 0 \cdot 002$ has been used.

It should be noted however that with quantities of silver nitrate below $1 \cdot 5$ gm. it was more difficult to get constant result, low rates of reaction being sometimes obtained. It is believed that this effect is due to impurity in the benzyl chloride, but it is hoped to investigate the matter further.

Effect of Speed of Shaking.

A few experiments were made to see whether the change in the speed of shaking has any effect on the velocity of reaction. The speeds employed were 180, 120 and 75 revolutions per minute of the shaker. The general experimental technique was the same as described before. The results are given in the Tables IV and V.

It will be seen from the results that change in the speed of shaking within limits has no effect on the velocity of reaction.

Effect of Water.

It has already been stated that moisture inhibits the reaction to a considerable extent. A few experiments were carried out to study quantitatively the effect of water on the rate of reaction. Distilled water was added to benzyl chloride before the reaction. Otherwise the experimental procedure was the same as before.

The systems studied were

Benzyl chloride	Silver nitrate	H_2O
5 c.c.	2 gm.	0·01 c.c.
5 ,,	2 ,,	0·05 ,,
5 ,,	2 ,,	0·10 ,,

TABLE IV.

Results with Variation in the Shaker Speed.

AgNO$_3$ initial	Benzyl chloride	Time	No. of shaker revolutions per mt.	AgNO$_3$ left gm.	AgNO$_3$ used gm.	K
1 gm.	5 c.c.	2·5 mts.	180	0·62	0·38	0·0017
			120	0·56	0·44	0·0023
			75	0·65	0·35	0·0017
1 gm.	5 c.c.	5 mts.	180	0·37	0·63	0·0018
			120	0·25	0·75	0·0024
			75	0·31	0·69	0·0021
1 gm.	5 c.c.	10 mts.	180	0·05	0·95	0·0020
			120	0·03	0·97	0·0022
			75	0·05	0·95	0·0020

TABLE V.

Results with Variation in the Shaker Speed.

AgNO$_3$ initial	Benzyl chloride	Time	No. of shaker revolutions per mt.	AgNO$_3$ left gm.	AgNO$_3$ used gm.	K
1 gm.	10 c.c.	2·5 mts.	180	0·60	0·40	0·0020
			120	0·62	0·38	0·0017
			75	0·58	0·42	0·0022
1 gm.	10 c.c.	5 mts.	180	0·33	0·67	0·0020
			120	0·28	0·72	0·0022
			75	0·30	0·70	0·0021
1 gm.	10 c.c.	10 mts.	180	0·07	0·93	0·0019
			120	0·04	0·96	0·0021
			75	0·09	0·91	0·0018

The results are shown graphically in Fig. 4. The effect of 0·01 c.c. of water on the reaction of 5 c.c. of benzyl chloride (0.18% by weight) is marked. This effect increases with the amount of water added.
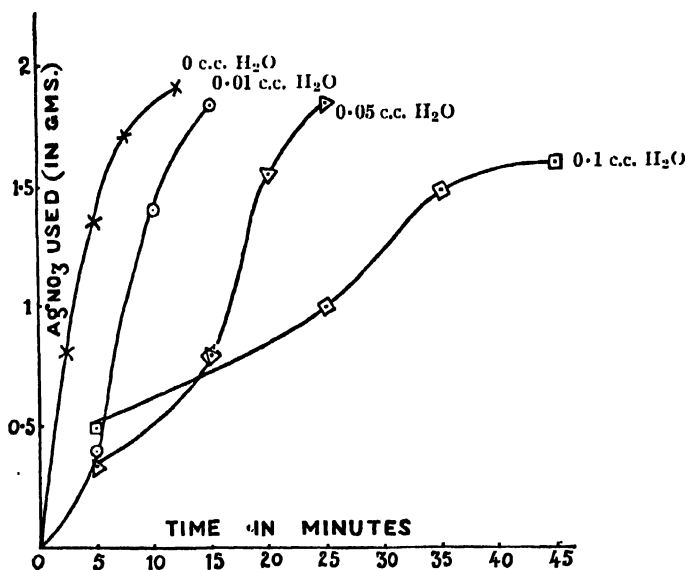
FIG. 4. Effect of H_2O.

Benzyl Chloride		AgNO₃	+ H₂O	
×	5 c.c.	+ 2 gms.	+ 0 c.c.	
⦿	,,	+ ,,	+ 0·01 c.c.	
◮	,,	+ ,,	+ 0·05 c.c.	
◻	,,	+ ,,	+ 0·1 c.c.	

Summary.

(1) The kinetics of the reaction between benzyl chloride and solid silver nitrate has been studied in the absence of solvents and diluents.

(2) The reaction is independent of the amount of benzyl chloride, but is proportional to the surface of silver nitrate present.

(3) The reaction has been studied with particles of three different sizes, and it has been shown that all the experimental results can be reproduced by the kinetic equation derived on the assumption that the rate of reaction depends only on the surface of silver nitrate present.

(4) The velocity of reaction is independent of the speed of shaking.

(5) Water inhibits the reaction; the effect of 0·18% by weight of benzyl chloride taken is marked and this effect increases with the amount of water added.

THE RAMAN SPECTRA OF DIOXANE AND TETRALIN.

By C. S. Venkateswaran.

(From the Department of Physics, Indian Institute of Science, Bangalore.)

1. *Introduction.*

In spite of the fact that organic compounds generally give rather compli-cated Raman spectra, the extensive researches in this field carried out in the last few years have enabled, in many cases, the modified lines to be classified into groups which can be identified as due to certain characteristic groups or chemical bonds in the molecule. In order to interpret the results in relation to the molecular structure, it is important to obtain the Raman spectra of the compounds studied as completely as possible. The simple ring com-pounds repeatedly investigated by several workers include benzene, cyclo-hexane and naphthalene. But comparatively little work has been done in other cyclic compounds possessing a somewhat similar structure. The present paper gives the results obtained by the author with dioxane and tetralin.

Explanation of Symbols.

Symbol	Wavelength	Wavenumber
a	3650	27358
b	3655	27353
d	4047	24705
e	4078	24516
f	4339	23039
g	4348	22995
h	4358	22938

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C. S. Venkateswaran

TABLE I.

The Raman frequencies of Dioxane from 4047 Å. U.—5100 Å. U.
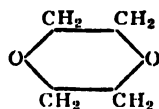
No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
	λ	ν		Without filter	With filter	
1	4062·5	24609	2779	1	..	a
2	4067·3	24580	2773	0	..	b
3	4076·6	24523	2863	4	..	a
4	4086·8	24462	243	2	..	d
5	4092·7	24427	2961	6b	..	a
6	4099·5 (double)	24387	$\overline{1449}$	3b	1	h
7	4111·7	24314	3075	1	..	a
8	4116·6	24285	421	0	..	d
9	4118·5	24273	433	3	..	d
10	4127·6	24220	485	5	..	d
11	4140·1	24147	$\overline{1209}$	0	..	h
12	4148·7	24097	419	0	..	e
13	4156·8	24050	466	0b	..	e
14	4188·5	23868	837	8sh	2	d
15	4191·1	23854	851	1sh	..	d
16	4204·7	23771	$\overline{835}$	3	1	h
17	4207·7	23759	946	0	..	d
18	4220·2	23689	1016	8	0	d
19	4221·4	23682	834	2	..	e
20	4237·1	23596	1109	3	..	d

TABLE I (*contd.*)

No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
	λ	ν		Without filter	With filter	
21	4240·3	23576	1129	3	..	*d*
22	4255·0	23195	1210	0	..	*d*
23	4256·8	23185	1221	5	0	*d*
24	4272·5	23399	1306	6	0	*d*
25	4277·4	23372	1333	2	..	*d*
26	4291·1	23297	1219	1	..	*e*
27	4297·8	23261	1441	6	0	*d*
28	4306·0	23249	1455	0	..	*d*
29	4307·9	23207	1299	0	..	*e*
30	4393·0	22757	181?	0	..	*h*
31	4441·0	22512	426	0	0	*h*
32	4442·3	22504	431	2	2	*h*
33	4451·6	22458	480	4	3	*h*
34	4523·2	22102	836	10*sh*	8	*h*
35	4525·6	22090	848	0	0	*h*
36	4535·2	22044	2662	1	..	*d*
37	4546·7	21988	2718 } 950 }	3 0	0	*d* *h*
38	4554·5	21950	2756	0	..	*d*
39	4560·3	21922	2783 } 1006 }	6	4	*d* *h*
40	4576·3	21846	2860 } 1109 }	8	2	*d* *h*
41	4582·2	21818	2888 } 1120 }	3	1	*d* *h*

TABLE I (concld.)

No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
	λ	ν		Without filter	With filter	
42	4597·7	21744	2962	10	1	*d*
43	4600·8	21729	1209	1	..	*h*
44	4603·4	21717	1221	6	4	*h*
45	4616·8	21654	2863	3	..	*e*
46	4621·4	21632	3074 ⎫ 1306 ⎭	8	4	*d* *h*
47	4626·2	21610	1328	2	1	*h*
48	4640·3	21544	2973	3(*b*)	..	*e*
49	4650·8	21496	1442	8	6	*h*
50	4652·6	21487	1451	0	0	*h*
51	4929·6	20279	2659	1	1	*h*
52	4944·5	20220	2718	1	1	*h*
53	4960·7	20153	2785	3	2	*h*
54	4980·9	20071	2867	8	6	*h*
55	4986·1	20050	2888	2	1	*h*
56	4991·6	20027	2968	1	0	*g*
57	5006·0	19970	2963	8*b*	8	*h*
58	5032·8	19864	3074	1	0	*h*

2. Experimental Arrangements and Results.

The experimental set up was the same as that previously used in the investigations of the author.[1] The two liquids used in this investigation were those used for the measurements of dipole moments at the Institute

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[1] Venkateswaran, C. S., *Proc. Ind. Acad. Sci.*, 1935, 1, 850.

TABLE II.

The Raman frequencies of Tetralin from
4047 Å. U.—5100 Å. U.

| CH   CH |
| CH$_2$\   /CH |
| C   C |
| CH$_2$\ /CH$_2$ |
| CH$_2$ CH$_2$ |

| No. | Raman lines | | $\Delta\nu$ in cm.$^{-1}$ | Intensity | | Assignment |
	λ	ν		Without filter	With filter	
1	4071·7	24553	153	6	1	d
2	4089·1	24448	258	2	0	d
3	4095·5	24410	2911	1	..	b
4	4104·6	24356	1418	0	..	h
5	4117·6	24279	427	2	0	d
6	4119·9	24266	440	2	..	d
7	4123·2	24216	460	0	..	d
8	4130·8	24202	504	1	..	d
9	4139·9	24148	557	0	..	d
10	4143·8	24128	578	3	..	d
11	4164·6	24005	701	1	..	d
12	4168·1	23985	721	8	2	d
13	4176·5	23946	760	0	..	d
14	4181·8	23906	800	1	..	d
15	4184·5	23891	815	2	..	d
16	4200·8	23798	908	2	..	d
17	4214·0	23724	982	0	..	d
18	4223·2	23672	1034	10	3	d
19	4228·0	23645	1061*	0	..	d

* An asterisk mark against some of the frequency numbers shows that they possess a doublet structure.

TABLE II (*contd.*)

No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
	λ	ν		Without filter	With filter	
20	4239·0	23584	1122	0	..	*d*
21	4245·8	23516	1160	1	..	*d*
22	4253·1	23506	1200	8	2	*d*
23	4258·4	23476	1230*	0	..	*d*
24	4268·2	23421	1285	3	..	*d*
25	4277·0	23369	1336	3	..	*d*
26	4279·5	23360	$\overline{1}22$	0	2	*h*
27	4285·7	23327	1379	2	..	*d*
28	4293·8	23278	1427	6	1	*d*
29	4298·4	23258	1448	1	..	*d*
30	4307·9	23207	1499	0	..	*d*
31	4309·7	23197	$\overline{2}59$	1	2	*h*
32	4322·0	23130	1575	0	..	*d*
33	4326·6	23106	1600	8	2	*d*
34	4329·3	23092	154	4	4	*h*
35	4387·9	22784	154	5	8	*h*
36	4408·1	22680	258	3	4	*h*
37	4409·9	22670	268	..	0	*h*
38	4440·0	22511	427	3	6	*h*
39	4443·1	22500	438	..	0	*h*
40	4446·0	22486	457	1	4	*h*

* An asterisk mark against some of the frequency numbers shows that they possess a doublet structure.

TABLE II (*contd.*)

No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
				Without filter	With filter	
41	4455·4	22438	505	2	5	*h*
42	4467·0	22380	558	0	1	*h*
43	4470·5	22363	575	3	4	*h*
44	4495·0	22241	697	1	1	*h*
45	4499·6	22218	720	8	10	*h*
46	4508·1	22176	762	0	0	*h*
47	4515·8	22138	800	0	1	*h*
48	4518·7	22124	811	1	2	*h*
49	4537·0	22035	903.	..	0	*h*
50	4544·1	22004	1035	0	0	*f*
51	4552·7	21959	1035 ⎫ 979 ⎭	0	2	*g, h*
52	4560·3	21903	1035	8	10	*h*
53	4570·3	21874	1061*	3	2	*h*
54	4576·9	21843	2863	1	..	*d*
55	4584·5	21806	2902	1	..	*d*
56	4590·5	21778	2930 ⎫ 1160 ⎭	4*b*		
57	4591·0	21762	2913	0	0	*d*
58	4601·4	21726	1202	5	6	*h*
59	4607·0	21704	1234	0	1	*h*
60	4617·1	21653	3052 ⎫ 1285 ⎭	6	3	*d, h*
61	4627·8	21602	1336	1	2.	*h*

TABLE II (*concld.*)

No.	Raman lines		$\Delta \nu$ in cm.$^{-1}$	Intensity		Assignment
	λ	ν		Without filter	With filter	
62	4636·3	21563	1383	1	2	*h*
63	4647·4	21511	1427	6	6	*h*
64	4652·2	21489	1449	1	2	*h*
65	4662·8	21440	1498	0	0	*h*
66	4680·8	21362	1576	2	2	*h*
67	4685·1	21339	1599	3	4	*h*
68	4980·8	20071	2867	3	3	*h*
69	4989·8	20035	2903	1	1	*h*
70	4993·7	20020	2918	0	0	*h*
71	4998·3	20001	2937	6	6	*h*
72	5018·7	19920	3008	1	1	*h*
73	5025	19892	3046	10	10	*h*

and the author's thanks are due to Dr. M. A. Govinda Rau for kindly permitting the use of the extra-pure substances which he had carefully prepared for his work. In order to facilitate the proper assignment of the excited lines, spectrograms were taken with and without filters. An alcoholic solution of *p*-nitro-toluene was found to be an efficient filter which cut off almost completely the 4046 radiations while transmitting the 4358 radiations of the source with maximum intensity. For dioxane, an alkaline solution of *o*-cresolpthalin helped to clear up the 4916 region considerably. The filter was contained in an outer jacket surrounding the experimental tube as in Fig. 1. It would be of interest to mention that tubes with almost perfectly flat ends could be easily made in pyrex glass with the outer tube fused to the bottom and supported with a few spokes dug into its walls at the top. In order to prevent the evaporation of the filter solution when the tube was placed close to the arc, the top end was closed with plaster of Paris through which two small tubes were passed for the introduction of the solution. The

<div align="center">

TABLE III.

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The intensities given in the brackets are for the 4358 excitations.

Dioxane		Tetralin			Cyclohexane	Benzene
Author	Villars	Author	Mukerji	Bonino and Cella	Krishnamurti	Grassman and Weiler (Incomplete)
181(0)?	..	151(8)	162(3)	158(4)	..	..
243(1)	..	259(4)	265(2)	265(3)	381($\frac{1}{2}$)	
	291(0)	268(0)	..	..	..	
425(0)	..	427(6)	430(4)	432(2)	425(1)	404(1)
		439(0)	..	..		
433(2)	434(00)	459(4)	..	..		
482(4)	519(00)	505(5)	511(3)	513(3)		
		558(1)	561(2)	567(2)		
		577(4)	582(3)	585(3)		607(8)
		699(1)	699(2)	..	695(0)	692(1)
		720(10)	723(5)	724(4)		
		761(0)	752(1)	759(1)		781(0)
837(8)	837(4)	800(1)	..	..	804(10)	802(0)
849(1)	..	814(2)	814(2)	815(2)		824($\frac{1}{2}$)
			839(0)	848(0)		849(4)
		905(0)				
946(0)	..	980(1)	..	..		984(2)
						992(15)
1012(6)	..	1035(10)	1037(7)	1038(5)	1028(8)	1031(1)
		1063(3)	..	..		
1109(3)	..	1122(0)	..	..		
	1117(1)					
1124(3)		1163(4)	1174(0)	1160(1)	1156(1)	1176(4)
1209(1)	..	1201(6)	1205(6)	1204(4)		
1221(5)	1214(1)	1232(1)	..	..	1266(5)	
		1285(3)	1283(1)	1283(2)		1285(0)
1306(8)	..	..	..	..	..	..
1328(2)	..	1336(2)	..	1310($1\frac{1}{2}$)	1344($\frac{1}{2}$)	1326($\frac{1}{2}$)
1444(8)	1442(2)	1427(6)	1433(3)	1433(3)	1444(5)	1403(2)

TABLE III (contd.)

Dioxane		Tetralin			Cyclohexane	Benzene
Author	Villars	Author	Mukerji	Bonino and Cella	Krishnamurti	Grassman and Weiler (Incomplete)
1451(1)	..	1448(2)	1458(5)	1456(3)		1449($\frac{1}{2}$)
		1499(0)	..	..		1480(0)
		1575(2)	1582(3)	1583(3)		1585(12)
		1600(4)	1602(3)	1602(3)		1606(8)
2660(1)	..	..	..	..	2662(1)	2615(2)
2718(1)	2720(00)	..	..	..		
2784(3)	..	'..	..	..		
2863(8)	2864(3)	2865(4)	2865(4)	2862(2$\frac{1}{2}$)	2852(8)	..
2888(2)	..	2902(1)	..	..	2889(1)	..
2968(10)b	2967(3)	2937(6)	2910(4)	..	2922(8)	2949(4)
		3008(1)	..	..		3049(8)
3064(1)	..	3049(10)	3046(5)	3049(5)		3064(12)
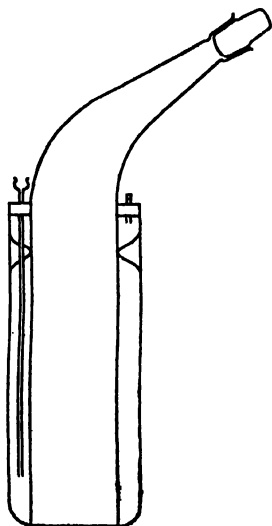
Fig. 1.

spectrograms were measured as usual and the results are tabulated in Tables I and II. The photographs are also reproduced in the accompanying plates. Dioxane was comparatively a poor scattering medium and an exposure of forty hours was required to bring out all the lines in its spectrum without any filter. Tetralin gave an intense picture at an exposure of nine hours; but in this case a continuous spectrum was also present.

3. Discussion of Results.

(a) *Dioxane.*—Except for a short report on the principal Raman lines by Villars,[2] this compound has not been studied in any great detail. From the point of view of the Raman effect this is an interesting compound, for it affords the unique example of a molecule possessing a structure similar

[2] Villars, *Jour. Amer. Chem. Soc.*, 1930, **52**, 4612.

to that of cyclohexane, but with two of the methylene groups replaced by oxygen atoms. As can be seen from the first column in Table III, the spectrum of dioxane consists of twenty-four lines of which fourteen have been observed for the first time. The author has been fortunate to be able to examine the cyclohexane plate of Krishnamurti[3] side by side with the latter and the comparison of the two reveals the following facts :—

(1) Both the compounds give only feeble wings accompanying the Rayleigh lines.

(2) All the important lines of cyclohexane appear more or less in the same position in dioxane.

(3) The region between 2863 and 2968 is covered by a band in both the cases which is attributed to the rotational spectrum accompanying the vibrational line due to C-H linkage.

(4) The wing structure of 1444 of cyclohexane is resolved into a close companion line in dioxane.

The outstanding differences in the two spectra are the following :—

(1) An intense line at 1306 (marked by the arrow in the accompanying plate) which is absent in cyclohexane, is one of the fundamental lines in dioxane and this is to be attributed to the oscillation of the group $O\begin{array}{c} \diagup CH_2 \\ \diagdown CH_2 \end{array}$.
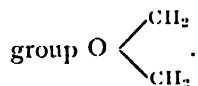
(2) The line due to the symmetrical oscillation of the carbons in cyclohexane at 801 is shifted to a higher frequency 837 in dioxane, and the lines due to C-H from 2852 and 2938 to 2863 and 2968 respectively.

(3) All the other lines belonging to C C or CH_2 are shifted in the opposite direction.

(4) The line at 425 which is assumed to be one of the characteristic lines of the cyclohexane nucleus, is resolved into three lines in dioxane.

(5) There is a group of lines present in dioxane at about 2750 which is not present in cyclohexane and they may be explained partly as arising from the combination of 1444 and 1306.

(6) The intense doublet due to C–H_2 in cyclohexane at 2922 and 2938 appears as a single broad line at 2968 in dioxane.

It is clear from the above that dioxane possesses a structure similar to that of cyclohexane and its vibrations are modified to some extent by the presence of the two oxygen atoms in the place of two CH_2 groups in the ring.

3 Krishnamurti, P., *Ind. Jour. Phys.*, 1932, **6**, 543.

The presence of a weak line at 3064 which is characteristic of the aromatic linkage shows besides that it represents the transition between the aromatic and the aliphatic ring compounds.

(b) *Tetralin.*—This has been studied in great detail first by Bonino and Cella[4] and quite recently by Mukerji.[5] These authors have pointed out the great similarity of its spectrum to those of cyclohexane and naphthalene. The results given by them are in fair agreement with those of the author. As can be seen from Table III new lines have been observed at 268, 439, 459, 800, 905, 980, 1063, 1122, 1232, 2902 and 3008. The frequency shifts of cyclohexane and of benzene[6] are also given for comparison. The new points that have been observed by the author in the spectrum of tetralin besides those reported by the previous investigators, are the following :—

(1) The line at 1440 due to the deformation oscillation of CH_2 has been shifted slightly to a shorter wavelength and has been followed by a companion as in dioxane.

(2) The line at 425 has been split up into three lines as in dioxane.

(3) The continuous spectrum between 2865 and 2937 in cyclohexane and dioxane is present weakly also in tetralin and thus appears to be characteristic of the molecules of the cyclohexane type.

(4) The wings accompanying the Rayleigh lines are as prominent as in benzene.

(5) The important lines in the benzene spectrum at about 1600 due to $C=C$ and above 3000 due to the aromatic CH appear also in tetralin.

(6) The line at 3016 shows a prominent wing on either side.

This similarity to the spectrum of cyclohexane on the one hand and of benzene on the other suggests that the molecule of tetralin is made up of one completed cyclohexane nucleus and one incomplete benzene nucleus.

In conclusion the author wishes to record his thanks to Sir C. V. Raman for his interest in the work.

Summary.

The Raman spectra of dioxane and tetralin have been obtained using the filter technique. The spectrum of dioxane consists of twenty-four lines of which fourteen are reported for the first time and resembles closely that of cyclohexane. Tetralin has also given eleven new lines. The results obtained are discussed with reference to the structure of the molecules.

[4] Bonino and Cella, *Atti. Acad. Lincei*, 1931, **13**, 784; also 1932, **15**, 572.

[5] Mukerji, *Phil. Mag.*, 1935, **19**, 1079.

[6] Grassman and Weiler, *Zeit. fur Phys.*, 1933, **86**, 314.
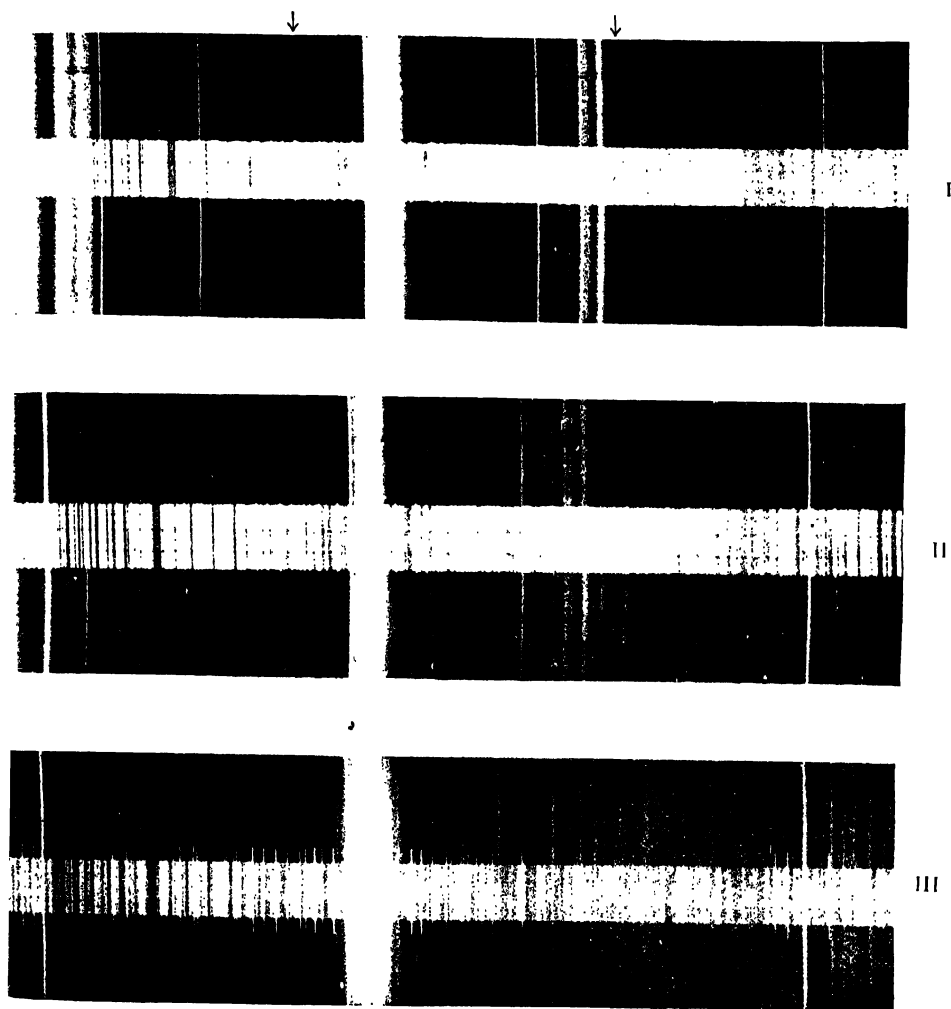
I. Dioxane without filter.   II. Dioxane with *o*-cresolthalin filter.
III. Tetralin with *p*-nitrotoluene filter.

THE RAMAN SPECTRUM OF HEAVY WATER.

By R. ANANTHAKRISHNAN.

(*From the Department of Physics, Indian Institute of Science, Bangalore.*)

1. *Introduction.*

THE Raman spectrum of heavy water was first photographed by Prof. R. W. Wood[1] who used in his investigations two samples of heavy water, one 18% and the other 80%. From his experiments, Wood arrived at the conclusion that the molecules containing one atom of heavy hydrogen (HDO) give a band $\Delta \nu = 2623$ cm.$^{-1}$ while those containing two atoms of heavy hydrogen (D$_2$O) give a band $\Delta \nu = 2517$ cm.$^{-1}$; ordinary water gives a band $\Delta \nu = 3445$ cm.$^{-1}$ Prof. Wood also remarked in his paper that he was unable to find any structure for these bands. In the case of the vapour, Wood got a line $\Delta \nu = 2674$ cm.$^{-1}$ for HDO and $\Delta \nu = 2601$ cm.$^{-1}$ for D$_2$O. Independently of Wood, Rank, Larsen and Bordner[2] photographed the Raman spectrum of heavy water vapour, and they found a line $\Delta \nu = 2718$ cm.$^{-1}$ for HDO and $\Delta \nu = 2666$ cm.$^{-1}$ for D$_2$O. Prof. Wood used in his work the resonance line 2536 Å of the mercury arc as the exciting line, while the latter authors used $\lambda = 4046$ Å as the exciting line. All the workers complain of the strong fluorescence of the vapour as well as of the liquid, which gave an undesirable background almost blotting out the Raman bands. However, Wood found that the fluorescence of the liquid disappeared after a few hours' exposure to the light of the mercury arc.

Judging from the close similarity in the properties of H$_2$O and D$_2$O, it appeared that the results of the above-mentioned authors are necessarily incomplete. The papers on the Raman spectrum of water are legion, and the majority of them seem to agree that the principal band of water consists of three components of which the central one $\Delta \nu = 3445$ cm.$^{-1}$ is the strongest. Apart from this a number of low frequency bands of lesser intensity have been noticed in the Raman spectrum of H$_2$O to which we shall have occasion to refer in the course of the paper. Special mention may, however, be made of the feeble band at 1650 cm.$^{-1}$ which has been observed as a line in the case of the vapour.[3]

[1] R. W. Wood, *Phys. Rev.*, 1934, **45**, 392.
[2] D. H. Rank, K. D. Larsen and E. R. Bordner, *Jour. Chem. Phys.*, 1934, **2**, 464.
[3] H. L. Johnson and M. K. Walker, *Phys. Rev.*, 1932, **39**, 535.

Theoretically, the computation of the fundamental frequencies of a non-linear triatomic molecule of the type AB_2 has been carried out recently by Van Vleck and Cross,[4] who give for the frequencies of vibration of the H_2O molecule, $\omega_1 = 3520$ cm.$^{-1}$, $\omega_2 = 3560$ cm.$^{-1}$ and $\omega_3 = 1660$ cm.$^{-1}$ Using the same molecular constants, Topley and Eyring[5] find for the frequencies of vibration of the HDO and D_2O molecules the following values :

	ω_1	ω_2	ω_3
HDO ..	3560 cm.$^{-1}$	2600 cm.$^{-1}$	1450 cm.$^{-1}$
D_2O ..	2580 cm.$^{-1}$	2590 cm.$^{-1}$	1250 cm.$^{-1}$

A calculation by Bonner[6] gives for the fundamental frequencies of the H_2O and D_2O molecules respectively

	ω_1	ω_2	ω_3
H_2O ..	3899 cm.$^{-1}$	3807·5 cm.$^{-1}$	1651·5 cm.$^{-1}$
D_2O ..	2865·4 cm.$^{-1}$	2764·7 cm.$^{-1}$	1209·7 cm.$^{-1}$

It might be pointed out that all the three frequencies are active in the Raman effect as well as in the infra-red, although ω_1 is relatively the least active in the Raman effect.

2. *Experimental.*

In the present work, a sample of 50 grams of 99·2g/100g D_2O (d^{20}_4 1·1049) supplied by the Norsk Hydro-Elektrisk Kvælstofaktieselskab was employed. The liquid was transferred into a thick-walled pyrex bulb of about 100 c.c. capacity attached to a Wood's tube of about 2 cms. diameter and 15 cms. length. The observation end of the tube was closed by fusing on a flat pyrex window in the usual way. After evacuating the system, the liquid was distilled into the tube and washed back a number of times, till finally the Wood's tube was filled with the dust-free liquid. The tube was suitably painted and illuminated by the light of a quartz mercury lamp concentrated on it by means of a 6-inch condenser. The scattered light was focussed on the slit of a Hilger two-prism spectrograph. When the alignment was perfect, the principal Raman bands could be seen visually with considerable

[1] J. H. Van Vleck and P. C. Cross, *Jour. Chem. Phys.*, 1933, 1, 350, 357.

[5] B. Topley and H. Eyring, *Jour. Chem. Phys.*, 1934, 2, 220.

[6] L. G. Bonner, *Phys. Rev.*, 1934, 46, 458.

brilliancy. Fluorescence was completely absent, and the continuous spectrum practically non-existent. Using Ilford Golden Iso-Zenith plates (H & D 1400), the spectrum could be photographed in all its salient features with an exposure of 10 hours. Longer exposures were tried to detect the presence of fainter bands, if any.

For the sake of comparison the Raman spectrum of ordinary water was also photographed with the same instrument. The water was first purified by distillation from $KMnO_4$ and $Ba(OH)_2$ respectively, and afterwards rendered dust-free by vacuum distillation in the usual way. The Wood's tube, as before, was of pyrex glass with fused-on end-window.

In order to determine the frequency shifts of the Raman bands, an iron arc comparison spectrum was taken in all cases, partially overlapping with the Raman spectrum.

3. Experimental Results.

(a) *Raman Spectrum of D_2O.*—We shall first consider the Raman spectrum of D_2O. Fig. 2 in the plate shows three broad and intense bands, which are due respectively to the excitation by the 3650 Å, 4046 Å and 4358 Å lines of the mercury arc. Even a casual examination shows that each of these bands consists of three components, of which the component with the largest frequency shift is the faintest. It should, however, be remarked that the 3650 Å excitation really arises from a group of lines all of which are of comparable intensity and hence there is a certain amount of confusion in the corresponding Raman band. Similarly, overlapping occurs between the Raman band excited by the 4046 Å and 4077 Å lines. In order, therefore, to gain a true insight into the structure of the band, we have to confine our attention to the band excited by the 4358 Å line whose companions are relatively very much feebler. The main features of this band are,

    (i)   it extends roughly over a range of 400 wave numbers ;

   (ii)   it consists of three distinct components which are fairly but not sharply resolved from one another ;

   (iii)   the central component is the brightest ; the component with the lowest frequency shift comes next in order of intensity, while the component with the largest frequency shift is comparatively feeble.

The next characteristic feature of the spectrum is the presence of a sharp band shifted by about 1230 cm.$^{-1}$ from the exciting line. This band is very clearly visible in the 4358 as well as in the 4046 excitations. Careful examination of the plate shows a faint companion towards the shorter wavelength side of this band in the 4358 excitation.

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Thirdly, the spectrum shows a strong band close to the exciting line, sharp towards the shorter wavelength side, and shading off towards the side of longer wavelength. This band extends from about 130 cm.$^{-1}$ to 240 cm.$^{-1}$ with the intensity maximum at about 175 cm.$^{-1}$

The following table gives the classification of the Raman spectrum of D_2O. The results of other authors are also added for comparison :—

TABLE I.

	Author				Wood	Rank, Larsen & Bordner
Exciting lines	4358	Int.	4046	Int.	2536	4046
Raman lines cm.$^{-1}$	130—240	st.				
	1110	ff.				
	1250	m.	1221	m.		
	2363	st.	2358	st.		
	2515	v.st.	2507	v.st.	2517 (liq.)	
	2662	m.	2680	m.	2601 (vap.)	2666 (vap.)

st.=strong ; v.st.=very strong ; m.=medium ; ff.=very faint.

The above set of measurements were made on one of the best of the author's plates, and are correct to ±5 wavenumbers. For the frequency shifts of the components of the principal band, the 4358 Å excitation values are probably more accurate, while for the frequency shift of the other band we shall adopt the mean value $\Delta \nu = 1235$ cm.$^{-1}$

(b) *Raman Spectrum of H_2O.*— In view of the importance of a comparative study of the Raman spectra of H_2O and D_2O, it may not be out of place to point out the salient features of the Raman spectrum of H_2O. Special mention must be made in this connection to the recent work of Magat[7], Bolla[8], Hulubei[9], Cabannes and De Riols[10] and Ramakrishna Rao[11] on the Raman

[7] M. Magat, *Jour. de Phys.*, 1934, **5**, 346.

[8] G. Bolla, *N. Cimento*, 1932, **9**, 290; *Ibid.*, 1933, **10**, 101.

[9] Hulubei and Cauchois, *C.R.*, 1930, **192**, 1040; *Ibid.*, 1932, **194**, 1475.

[10] Cabannes and De Riols, *C.R.*, 1934, **198**, 30.

[11] I. R. Rao, *Proc. Roy. Soc.*, 1931, **130**, 489; *Ibid.*, 1934, **145**, 489; *Phil. Mag.*, 1934, **17**, 1113.

spectrum of H_2O. While Bolla, Cabannes and De Riols and Ramakrishna Rao claim that the principal band of water consists of three components whose frequency shifts are approximately 3200, 3400 and 3600 cm.$^{-1}$, Magat and Hulubei have not been able to find any trace of the third component with the largest frequency shift. In the Raman spectrum of water taken at room temperature (27° C.) [Fig. 3 in the plate], the author finds that the third component is very faintly present in the Raman band excited by the 4358 Å line of the mercury arc. It is true that this band happens to fall in a region in which the photographic plate is relatively little sensitive, but the time of exposure was adjusted to record it with sufficient intensity. The Raman band due to the 4046 Å excitation also shows a triple structure. The third component is apparently much stronger in this case which is undoubtedly due to the fact that the second component of the band due to the 4077 excitation happens to fall in almost the same position as the third component due to the 4046 excitation.

Besides the principal band, a faint band $\Delta \nu = 1650$ cm.$^{-1}$ has been noticed in the Raman spectrum of H_2O by all the above-mentioned authors except Rao. It should be pointed out that the 2536 excitation employed by them is particularly favourable for the observation of this band, since the above band excited by the 4046 Å line chances to fall in the 4358 region and the same due to the 4358 excitation would merge with the principal water band excited by the 4046 Å line. Magat, using a filter which transmits only the 4358 group has, however, been able to record this band. In the photograph reproduced in the plate, this band is faintly visible to the shorter wavelength side of the 4358 group.

Magat has reported a band of medium intensity with $\Delta \nu = 5100$ cm.$^{-1}$ However, no trace of this band could be detected in any of the author's plates.

Two other feeble bands with $\Delta \nu = 500$ cm.$^{-1}$ and $\Delta \nu = 750$ cm.$^{-1}$ have been reported by Magat, Bolla and Cabannes and De Riols. These bands are recorded on the author's plates as a broad structure extending over a region of about 500 wavenumbers.

In addition to these, Bolla and Magat have found a strong band with a frequency shift of about 170 cm.$^{-1}$ In fact the presence of such a strong low frequency band in the case of water was first noticed by Segre.[12] The existence of a similar band in the Raman spectrum of D_2O has been already remarked. This band is also intensely recorded on the author's plates in the case of H_2O. In fact, the 4046 excitation shows a faint but well-defined antistokes for this band.

[12] E. Segre, *Accd. Lincei, Atti.*, 1931, **13**, 929.

The following table gives the frequency shifts of the Raman bands of H_2O. The results of other authors are included for the sake of comparison :

TABLE II.

	Author		Magat		Bolla	Cabannes & De Riols	I. R. Rao
Exciting line	4046	4358	4358	2536	2536	2536	
Raman lines cm.$^{-1}$	134-236(st.)	134-243(st.)	152-225(m.)	175	60(f.) 172(st.)		
	..	464(f.)	600(ff.)	500	510(st.)	550	
	..	754(f.)		740	780(f.)	700	
	1665(f.)	..	1659(f.)	1659	1645(f)	1643	
	3231(st.)	3214(st.)	3221(st.)	2135 3200	2150(ff.) 3200(st.)	3224	3216(st.)
	3436(v.st.)	3428(v.st.)	3435(v.st.)	3400	3435(st.)	3436	3435(v.st.)
	3605(m.)	3600(f.)	..	..	3630(m.)	3625	3528(f.)
					3990(m.)		
			5090(m.)	5100			

st.=strong ; v.st.=very strong ; m.=medium ; f.-feeble ; ff.=very feeble.

4. Discussion of Results.

(a) *Modes of vibration of a non-linear triatomic molecule of the type* AB_2*.
—We know that a molecule of this type possesses three fundamental modes of vibration which can be diagrammatically represented as follows :
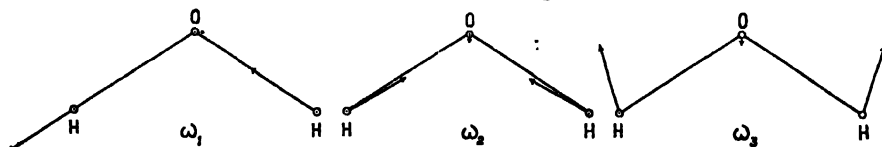
FIG. 1 (a). Normal vibration of H_2O molecule.
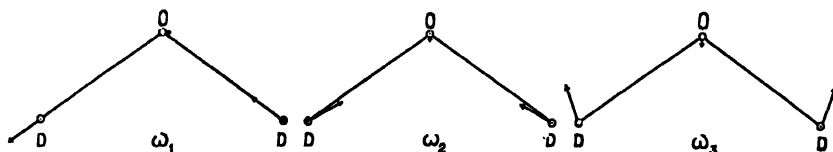
FIG. 1 (b). Normal vibrations of D_2O molecule.

* For discussion under this head the author is highly indebted to Mr. N. S. Nagendra Nath, to whom he wishes to acknowledge his thanks.

We may expect ω_2 and ω_3 to be more active in the Raman effect and ω_1 and ω_3 to be more active in the infra-red. In fact, the Raman spectrum of water vapour shows only two frequencies 3655 cm.⁻¹ and 1650 cm.⁻¹ which may be identified with ω_2 and ω_3 respectively. In the corresponding case of D_2O only one frequency $\Delta\nu = 2666$ cm.⁻¹ has been so far reported, but the author is quite certain that a careful examination of the Raman spectrum of D_2O vapour will reveal a line at about 1235 cm.⁻¹

The infra-red spectrum of heavy water vapour has been studied recently by Bartholomé and Clausius[13] who find the following frequencies :

	ω_1	ω_2	ω_3
H_2O ..	3756		1595
HDO ..	3720	2810	1380
D_2O ..	2775		1185

ω_2 is observed in the infra-red in the case of HDO, while it fails to appear in the case of the other two symmetrical molecules. The corresponding Raman data are summarised in the table below :

	ω_1	ω_2	ω_3
H_2O ..		3655	1650
HDO ..		2718 2671	?
D_2O ..		2666 2601	(1235)

It is very strange that the value of ω_3 as revealed in Raman spectrum is higher than the observed infra-red value both in the case of H_2O and D_2O.

Adopting the general potential energy function with 4 constants, if we represent the fundamental frequencies of the H_2O molecule by ω_1, ω_2 and ω_3, and those of the D_2O molecule by ω_1^*, ω_2^* and ω_3^* respectively, it can be shown that[14]

[13] E. Bartholomé and K. Clausius, *Naturwiss.*, 1934, **22**, 420; *Zeit. fur. Elek. Chem.*, 1934, **40**, 530.

[14] See Jenny E. Rosenthal, *Phys. Rev.*, 1934, **45**, 426.

$$\frac{\omega_1}{\omega_1{}^*} = \left[\frac{D}{H} \cdot \frac{O+2H\ \sin^2 a}{O+2D\ \sin^2 a}\right]^{\frac{1}{2}} \qquad .. \qquad .. \qquad .. \quad (1)$$

and $\quad \dfrac{\omega_2\ \omega_3}{\omega_2{}^*\omega_3{}^*} = \left[\dfrac{D^2}{H^2} \cdot \dfrac{O+2\ H}{O+2\ D}\right]^{\frac{1}{2}} \qquad .. \qquad .. \qquad .. \quad (2)$

where O, H, D stand respectively for the masses of the oxygen, hydrogen and deuterium atoms, and $2a$ is the valence angle subtended at the oxygen atom.

In deriving (1) and (2) it is assumed that the valency angle as well as the force constants remain unaltered when one passes from H_2O to D_2O. Bartholomè and Clausius have calculated from equation (1) the vibration frequency of the D_2O molecule assuming the frequency and the valence angle for H_2O, and have found good agreement between the observed and the calculated values. Equation (2), however, affords an independent check on these assumptions purely from Raman data. If we substitute the observed Raman frequencies in the left-hand side of equation (2) we get

$$\frac{\omega_2\ \omega_3}{\omega_2{}^*\omega_3{}^*} = \frac{3655 \times 1650}{2666 \times 1235} = 1 \cdot 83$$

The right-hand side of the equation works out as $1 \cdot 89$. The agreement is not unsatisfactory and shows that the force constants and valency angles are nearly but perhaps not quite identical in the two cases.

(b) *Structure of the Principal Raman band.*—The structure of the principal Raman band of water does not appear to have been satisfactorily accounted for, although numerous papers have been written over it.   The fact that the Raman band of D_2O also shows an exactly identical structure is not surprising in view of the close physical and chemical similarity of these two substances.   One important fact that deserves attention is that the frequency of the third component is very nearly identical with the frequency observed in the vapour state, being respectively 3600 and 3655 cm.[-1] in the case of H_2O and 2662 and 2666 cm.[-1] respectively in the case of D_2O.   In the case of water, Ramakrishna Rao[15] has put forward the theory that there exist three different types of molecules, H_2O, $(H_2O)_2$ and $(H_2O)_3$. Rao attributes the component of highest frequency observed in the Raman spectrum of water to the H_2O molecules, while the components of lower frequency are attributed respectively to the $(H_2O)_2$ and $(H_2O)_3$ molecules. Rao gives the following arguments in favour of his theory :

  (i)   the frequency of the third component of the Raman band is not
         very different from the frequency observed in the vapour state ;

  (ii)   as the temperature of water is raised the third component goes
         on gaining in intensity, while the first one goes on diminishing

15 I. R. Rao, *loc. cit.*

in intensity ; this is in all probability due to the breaking up of the higher polymers, evidence of whose existence in water is available from entirely different sources ;

(iii) the Raman spectrum of ice shows only the first two components, which are sharper than in the case of water and are shifted slightly towards the exciting line. This is because all the water molecules exist as double and triple molecules in the case of ice ;

(iv) the Raman spectrum of crystals containing water of crystallisation shows only the first or the first two components, but in no case the third.

Although the reasoning appears quite plausible, Sutherland[16] has pointed out that the assumption of the existence of triple molecules in water and ice is contrary to experimental evidence. He finds that the character of the Raman spectrum of ice taken at liquid air temperature is strikingly different from that taken at $0°$ C. The component of lowest frequency is much sharper and stronger at the lower temperature, which is explicable on Rao's theory only if we make the improbable assumption that even in the crystalline state a gradual polymerisation into $(H_2O)_3$ molecules takes place. X-ray observations[17] do not however show any change in the crystal structure of ice between $0°$ C. and $-183°$ C. Sutherland attributes the component of frequency 3200 cm.$^{-1}$ to $(H_2O)_2$ molecules, and postulates that the other two frequencies arise because of a doubling of the H_2O frequency from a condition of resonance degeneracy such as has been observed in the case of CO_2. "This is suggested by the fact that one of the other fundamental frequencies of water is known from infra-red and Raman spectra to lie near 1650 cm.$^{-1}$ so that its first overtone may fall close enough to the fundamental for these two levels to interact. As the temperature is lowered the position of the fundamental shifts considerably, as well as being more sharply defined, so that the interaction between the two levels may become negligible and only one frequency will be observed."

The explanation of Sutherland, however, appears rather far-fetched. The analogy with CO_2 appears difficult to understand. It need only be pointed out that whereas the frequencies 1285 and 1388 cm.$^{-1}$ of the components into which the fundamental frequency is split up in the case of CO_2 lie one on either side of the octave of the other fundamental frequency 668 cm.$^{-1}$, the author is unable to find any such relation in the case of either H_2O or D_2O. The investigation of the band structure of HDO would be of great interest in this connection.

[16] G. B. B. M. Sutherland, *Proc. Roy. Soc.*, 1933, **141**, 542
[17] See Barnes, *Proc. Roy. Soc.*, 1929, **125**, 670.

Recently Bernal and Fowler[18] have proposed a model of the water molecule from considerations of spectral and X-ray data, which has proved to be of great help in explaining either quantitatively or qualitatively several properties of water and ionic solutions. According to this model an H_2O molecule of radius $1\cdot4$ Å is surrounded by four others in a more or less regular tetrahedron. "This is the arrangement found in ice and necessarily follows from the quasi-tetrahedral angle of the H_2O molecule." As regards the arrangement of the molecules of H_2O in water, Bernal and Fowler postulate three chief forms of arrangement :—"Water I, tridymite-ice-like, rather rare, present to a certain degree at low temperatures below 4° C. ; water II, quartz-like predominating at ordinary temperatures ; water III, close-packed ideal liquid, ammonia-like predominating at high temperatures for some distance below the critical point at 374° C. These forms pass continuously into each other with change of temperature..... The sequence water I II–III is one of increasing rotatory and translatory molecular movement and of the consequent diminution of the dipole forces of cohesion of the liquid and relative increase of the Van der Waals component..... It is tempting to identify the 3200, 3400, 3600 (Raman) bands as corresponding respectively to the water structures I, II and III, but such a correspondence cannot be maintained until the nature of the transitions corresponding to these bands has been worked out."

We may here point out one significant fact of experimental observation, that while the frequency difference of the components of the band is about 200 cm.$^{-1}$ in the case of H_2O, this difference is only about 150 cm. ^{1} in the case of D_2O.

(c) *Band* $\Delta v = 500$ *cm.*$^{-1}$ —This diffuse band has been attributed by Magat to the frequency of hindered rotation or oscillation of the water molecules. However, this assignment would appear to be purely conjectural, and the origin of this band as well as of the band $\Delta v - 750$ cm.$^{-1}$ remains rather obscure. It is surprising that while Magat[19] has found that these bands disappear at about 40° C., Bolla[20] has quite recently reported that these bands persist in the Raman spectrum of water at all temperatures from 28° to 92°C.

(d) *Band* $\Delta v = 175$ *cm.*$^{-1}$—Segre who was the first to observe this strong band in the case of H_2O suggested that it might be due to the oscillation in the polymers of H_2O. He found that raising the temperature, which tends to reduce the number of associated molecules, decreased the intensity

[18] Bernal and Fowler, *Jour. Chem. Phys.*, 1933, 1, 515.
[19] M. Magat, *Jour. de Phys.*, 1934, 5, 347.
[20] G. Bolla, *N. Cimento*, 1935, 12, 243.

of this band. Magat has made an attempt to calculate the frequency of such an oscillation on the approximate assumption that it is analogous to the intramolecular vibration of a diatomic molecule, and employing the empirical formula of Morse[21] connecting the frequency ω_0 and the internuclear distance r_0

$$\omega_0 r_0^3 = 3000 \text{ Å}^3/\text{cm}.$$

Putting $r_0 = 2 \cdot 72$ Å (diameter of the H_2O molecule), we get $\omega_0 = 150$ cm.[-1] In view of the highly empirical nature of the procedure, the agreement seems satisfactory.

Alternatively, Magat has calculated the frequency of such an intermolecular vibration as 200 cm.[-1] by employing the model proposed by Bernal and Fowler, which value again falls in the observed range. However, as Magat himself has remarked, such a vibration cannot appear in the infra-red, since the total electric moment of a group of five molecules would on the average be zero.

The existence of a strong infra-red absorption for water at about 160 cm.[-1] has been found recently by Cartwright.[22] In a later note in *Nature*,[23] Cartwright has reported that D_2O also shows a strong infra-red absorption in practically the same region. These results fit in very well with the author's observation that the corresponding Raman band in the case of H_2O and D_2O are practically in the same position. Cartwright originally attributed the infra-red band in the case of H_2O to a hindered rotation of the water molecules. Support for the view that the molecules in a liquid exist in a quasi-crystalline state is forthcoming from divers sources. Recently Debye[24] has adduced strong evidence to show that the rotation of the dipole through 90° C. in the case of H_2O would produce a potential energy of about 10 kT. If we adopt this value and calculate the frequency of hindered rotation about the axis perpendicular to the plane of the molecule (assuming the oscillation to be approximately harmonic), we get a value of 200 cm.[-1]

However, such a simple calculation leaves unexplained the observed fact that the frequency of the band is practically the same in the case of H_2O and D_2O. If the origin of the band is to be sought for in the rotation of the individual dipoles, it follows that the frequency of the band ought to be affected by a factor $\sqrt{2}$ when we pass from H_2O to D_2O. Cartwright has therefore modified his original statement, and has attributed the infra-red

[21] P. M. Morse, *Phys. Rev.*, 1929, **34**, 57.

[22] C. H. Cartwright, *Nature*, 1935, **135**, 872.

[23] C. H. Cartwright, *Nature*, 1935, **136**, 181.

[24] P. Debye, *Phys. Zeit.*, 1935, **36**, 100, 193.

absorption band at 160 cm.$^{-1}$ to the hindered translation of the liquid molecules, in which case the change in frequency from H_2O to D_2O would be small, in conformity with the experimental result. We may point out that the difficulty of attributing the band to hindered rotation of the molecules might be overcome, if it be assumed that the rotating entities are not the individual molecules, but the polymers of H_2O and D_2O respectively. The knowledge of the state of polarisation of the band would most probably throw light on its origin ; measurements are now on hand for this purpose.

In conclusion, the author wishes to record his respectful thanks to Professor Sir C. V. Raman for his kind interest and helpful guidance in the course of the present investigation.

5. *Summary.*

The Raman spectrum of heavy water (D_2O) photographed with a Hilger two-prism spectrograph shows a strong band with three imperfectly resolved components whose frequency shifts are 2363, 2515 and 2662 cm.$^{-1}$ Besides, a sharp band with a frequency shift of 1235 cm.$^{-1}$ with a feeble companion at about 1110 cm.$^{-1}$ has been recorded, and a strong low frequency band $\Delta \nu = 175$ cm.$^{-1}$ The triple structure of the principal band as well as the existence of the other two bands has been noticed for the first time. A comparative study of the Raman spectra of H_2O and D_2O has been made and many points of similarity noticed. Purely from Raman data, it is shown that the valency angles and force constants do not alter much as one passes from H_2O to D_2O. It is pointed out that the triple structure of the principal band is probably due to the fact heavy water is also polymerised similar to H_2O. The low frequency band appears to be intimately connected with the nature of the liquid state.
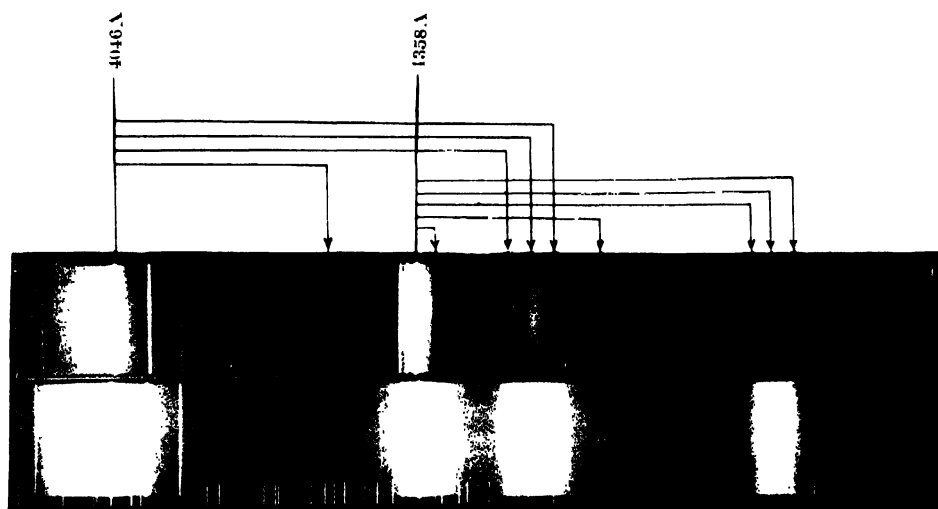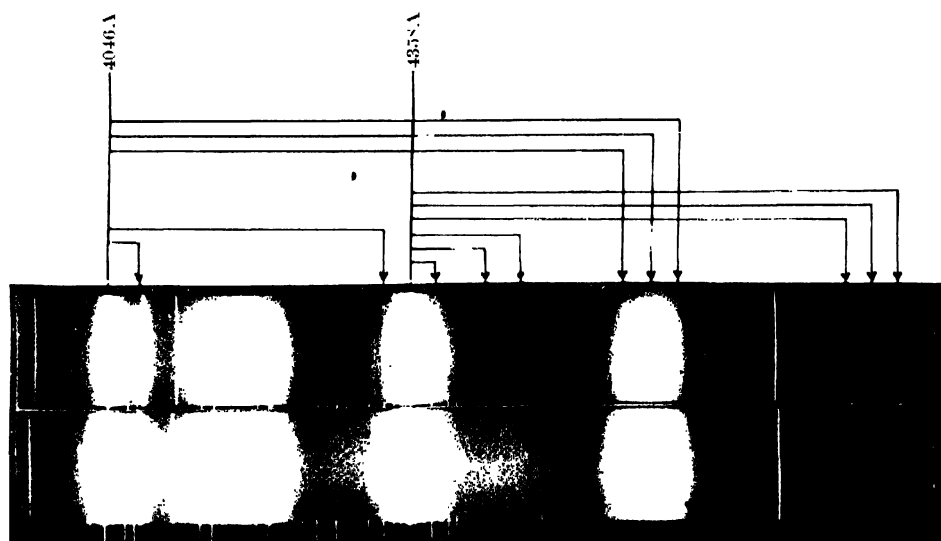
FIG. 2.   Raman Spectrum of D_2O.

FIG. 3.   Raman Spectrum of H_2O.

RAMAN SPECTRUM OF DEUTERIUM: I.

BY S. BHAGAVANTAM.

(From the Department of Physics, Andhra University, Waltair.)

1. *Introduction.*

IN two earlier papers (Bhagavantam, 1932), the author studied the Raman spectrum of ordinary hydrogen in all its aspects in great detail using a specially constructed high pressure gas tube as the container for the gas. It is felt that these investigations should be extended to the case of heavy hydrogen as of all the other molecules that are amenable to such studies, it comes next in simplicity. The necessity of making a complete experimental survey of the Raman spectrum in such a case so as to include all the important features such as the intensity relationships, polarisation characters and fine structure of the lines, need not be overstressed here. It may, however, be pointed out that ordinary and heavy hydrogen constitute the only two cases which may be expected to furnish unique experimental evidence on a variety of points in favour or otherwise of the existing theories of the Raman effect. This, of course, is intimately connected with the very low moments of inertia and the simplicity of structure of these molecules. The present paper is the first of a series dealing with the Raman spectrum of deuterium which the author proposes to publish and contains the preliminary results obtained in this direction.

2. *Experimental.*

Preparation of Deuterium.—The following procedure is adopted for preparing deuterium under high pressure. About 5 grammes of heavy water supplied as 99·5% pure by the Ohio Chemical and Manufacturing Company is further purified by slow distillation in an evacuated and sealed double bulb of pyrex glass. The water thus purified is transferred to a thin wall glass capsule. The glass capsule is sealed off and carefully inserted into a specially prepared steel bomb. About 6 grammes of sodium and a small steel hammer are introduced into the bomb which is then closed. Through a pin valve connected to the bomb all the enclosed air is removed thoroughly by means of an efficient oil pump, till a manometer connected in the circuit showed that any air that may have been left inside the bomb is only at a pressure of a fraction of a millimeter. The pin valve is then closed and the

steel hammer dropped, by suitably tilting the bomb, on to the water bulb. The bulb breaks and the water at once reacts with the sodium thus generating deuterium inside the bomb at a high pressure. The volume chosen for the bomb is such that the above quantities of heavy water and sodium have generated a pressure of about 100 atmospheres. The reaction is almost instantaneous and is accompanied by the evolution of a large quantity of heat. The total volume of the deuterium generated is about 3 litres at atmospheric pressure.

The gas is then allowed to cool and is transferred into the experimental tube designed for a study of the Raman effect in gases at high pressures and described by the author in the earlier papers already referred to. The connecting tubes used in transferring the gas and the experimental tube are thoroughly evacuated beforehand through pin valves. In this way the experimental tube is filled with fairly pure (nearly 100%) deuterium at a pressure of about 17 atmospheres.

Exposure.— Light from a six-inch quartz mercury arc is condensed by means of a large glass condenser on to the gas tube. The scattered light is focussed with a short focus lens on the slit of a Hilger 2 prism glass spectrograph of high light gathering power. Using a slit width of 0·05 mm. a continuous exposure of about 72 hours is found necessary to record the Raman spectrum with reasonable intensity. Golden Isozenith plates have been used for photographing the spectra.

3. Results.

Table I gives the various lines recorded and measured in the Raman spectrum of deuterium in the present investigation. The plate shows other feeble lines which are not included in the table and these will be measured and interpreted in subsequent communications after obtaining more intense photographs. Figs. (*a*) and (*b*) in the Plates are respectively the Raman spectrum of deuterium and a microphotometric record of the same. The transitions are given for some of the rotation lines that are easily seen in the reproductions. V.R. indicates the vibration Raman line excited by λ 4046. The microphotometric record is confined only to the rotation lines in the neighbourhood of λ 4358 and is intended to exhibit the approximate relative intensities of these lines. The most remarkable feature s the alternation of intensities, lines representing transitions between even rotational quantum numbers being stronger than those that represent transitions between odd rotational quantum numbers. The nearly equal intensity of the $0 \rightarrow 2$ and the $2 \rightarrow 4$ lines may also be noted and is of special significance.

TABLE I.

Raman Spectrum of Deuterium.

Wave-length	Approx. Rel. Intensity	Exciting line	Frequency shift	Quantum transition	
				J	n
4601·27	2	4046	2992·7	$\left\{\begin{array}{l}0 \to 0\\1 \to 1\\\text{etc.}\end{array}\right\}$	0→1
4484·13	1	4358	613·5	4 → 6	
4461·58	1	,,	530·8	3 → 5	
4438·69	5	,,	415·2	2 → 4	
4415·58	3	,,	297·4	1 → 3	
4392·74	5	,,	179·5	0 → 2	
4324·77	0	,,	−178·1	2 → 0	
4302·54	0	,,	−297·5	3 → 1	
4115·67	4	4046	414·9	2 → 4	
4096·02	3	,,	298·4	1 → 3	
4018·01	0	,,	−175·4	2 → 0	

4. Discussion of Results and their Comparison with Theory.

(a) *Frequency Shifts and the Molecular Constants.*—The Raman spectrum of deuterium at a pressure of 2·5 atmospheres has been investigated by Anderson and Yost (1935) recently using λ 2537 of mercury as the exciting radiation. These authors have reported two rotation lines having frequency shifts of 179·6 and 298·3 and a vibration line having a frequency shift of 2989·5. These figures are in satisfactory agreement with those obtained by the author. In the present investigation use is made of λ 4358 as the exciting radiation and it has been possible to record several other rotation lines and some antistokes lines as the gas is obtained at a much greater pressure. Hitherto there existed no direct experimental evidence for the molecular constants of deuterium. These have been derived by Urey and Teal (1935) only indirectly by making use of the data available for the H_2 and HD molecules with the help of the usual relations between the constants of

A7   F

isotopic molecules. Anderson and Yost have shown that these constants satisfactorily predict the positions of the two rotation lines observed by them in the Raman spectrum of deuterium. This agreement may be regarded as the first direct experimental evidence for the molecular constants of deuterium but cannot be considered complete as the anharmonic constants and the small correction terms do not make themselves felt appreciably until we reach large rotation quantum numbers. The fact that in the present investigation, five lines have been recorded and measured, enables us to make the comparison much more complete and the results provide a most satisfactory confirmation of the constants derived by Urey and Teal. The rotational energy of a diatomic molecule in a specified electronic state and zero vibrational state is given by the following equation

$$\frac{E_J}{hc} = \left[B_e - \frac{a_e}{2} + \gamma - \frac{\delta}{8} \right] J(J+1) + \left[D_e + \frac{\beta}{2} \right] J^2(J+1)^2 + F_e J^3(J+1)^3 \quad .. \ (1)$$

In this equation J is the rotational quantum number and B_e is the moment of inertia of the molecule in a state of vibration of infinitesimal amplitude and is related to B_o the moment of inertia of the molecule in the zero vibrational state by the equation

$$B_o = B_e - \frac{a_e}{2} + \frac{\gamma}{4} \text{ etc.} \tag{2}$$

The other constants are all small and enter only as correction terms. Assuming, in accordance with Urey and Teal, that $B_e = 30 \cdot 459$; $a_e = 1 \cdot 0858$; $\gamma = 0 \cdot 01713$; $\delta = 0 \cdot 00115$; $D_e = -0 \cdot 01121$; $\beta = 2 \cdot 39 \times 10^{-4}$, the rotational energies pertaining to the various J values are calculated from (1) and the frequency shifts of the Raman lines $0 \to 2$, $1 \to 3$, etc. are deduced. These are given in Table II along with the frequency shifts observed in the present investigation for comparison. Since the antistokes rotation lines and those excited by λ 4046 are somewhat weak, in giving the figures in Table II, only the lines excited by λ 4358 are taken into consideration.

TABLE II.

Comparison with Theory.

Transition	J : 0 → 2	1 → 3	2 → 4	3 → 5	4 → 6
Observed frequency	179·5	297·4	·415·2	530·8	643·5
Calculated frequency	179·1	297·7	414·9	530·3	643·5

The excellent agreement between the two sets of values up to the fifth rotation line must be regarded as a satisfactory confirmation of both the main molecular constants and the correction terms as well.

(*b*) *Relative Intensities of the Rotation Lines, Spins and Statistics of the Nuclei*. —From observations on the alternation of intensities in the α bands of deuterium, Lewis and Ashley (1933) have concluded that the nucleus of deuterium obeys the Einstein-Bose statistics, and that the spin cannot be zero. Murphy and Johnston (1934) working on the Fulcher spectrum of D_2 have concluded that the statistical weight of the symmetric states is twice that of the antisymmetric states. This would mean that the spin of the deuterium nucleus is 1. These results indicate that in the Raman spectrum of deuterium, unlike the case of ordinary hydrogen whose nuclei obey the Fermi-Dirac statistics, the lines representing transitions between even rotational quantum numbers should be stronger than those that correspond to transitions between odd rotational quantum numbers. This is most satisfactorily confirmed in the present investigation as may be seen from Figs. (*a*) and (*b*) in the Plates. The formulæ originally developed by Manneback (1930) and subsequently also given by Placzek (1934) for the relative intensities of the rotational Raman lines in the case of a diatomic molecule are made use of in calculating the intensities given in Table III. It is assumed that molecules with even rotational quantum numbers have a statistical weight twice as large as that possessed by those having odd rotational quantum numbers. The calculations are for a temperature of 30^c C. and assume that $B_0 = 29 \cdot 916$.

TABLE III.

Calculated Relative Intensities of the Rotation Lines.

P P Series : $J \rightarrow J - 2$.		R R Series : $J \rightarrow J + 2$	
Transition	Intensity	Transition	Intensity
$2 \rightarrow 0$	$0 \cdot 571$	$0 \rightarrow 2$	$1 \cdot 333$
$3 \rightarrow 1$	$0 \cdot 220$	$1 \rightarrow 3$	$0 \cdot 901$
$4 \rightarrow 2$	$0 \cdot 202$	$2 \rightarrow 4$	$1 \cdot 467$
		$3 \rightarrow 5$	$0 \cdot 407$
		$4 \rightarrow 6$	$0 \cdot 322$
		$5 \rightarrow 7$	$0 \cdot 020$

The figures are only relative and have no absolute significance. It is easily seen from the pictures that the relative intensities experimentally obtained are in qualitative agreement with the above figures. The fact that the lines $0 \rightarrow 2$ and $2 \rightarrow 4$ are to be expected to be of nearly the same intensity, both being more intense than $1 \rightarrow 3$ is beautifully confirmed in the microphotometric record of Fig. (*b*) in the Plates. The intensity distribution amongst the various lines is a very exceptional one and has not been observed in any of the Raman spectra so far studied.

(c) *The Vibration Line and Its Fine Structure.*—The vibration line $n : 0 \rightarrow 1$ should consist of several fine structure components arising from different molecules belonging to different rotational states. The spacing of these may be calculated using the constants given by Urey and Teal. In the present investigation, however, the resolution employed and the exposure given are sufficient only to bring out the strongest of these components and a detailed calculation of the positions and the relative intensities of these components is therefore postponed to a later communication. The strongest line should have a frequency shift of $2991 \cdot 2$* and the observed value of $2992 \cdot 7$ is in good agreement with this figure. Anderson and Yost have given $2989 \cdot 5$ for the frequency shift of this line.

5. *Summary and Conclusion.*

The paper describes the results of a study of the Raman spectrum of deuterium at a pressure of about 17 atmospheres using λ 4358 of mercury as the incident radiation.

Rotation lines having frequency shifts $\pm 179 \cdot 5$, $\pm 297 \cdot 4$, $415 \cdot 2$, $530 \cdot 8$ and $643 \cdot 5$ and a vibration line with a shift of $2992 \cdot 7$ have been observed. These shifts are in excellent agreement with and provide for the first time a direct experimental confirmation of the molecular constants given by Urey and Teal for the D_2 molecule.

The relative intensities of the rotation lines are in agreement with the fact that the deuterium nucleus has a spin of one unit and obeys the Bose-Einstein statistics. States characterised by even rotational quantum numbers are given a statistical weight twice as large as that of those having odd rotational quantum numbers.

The intensity distribution is in qualitative agreement with that predicted by the theory developed by Manneback for the rotational Raman scattering in diatomic molecules.

* See T. F. Anderson and D. M. Yost, *loc. cit.*
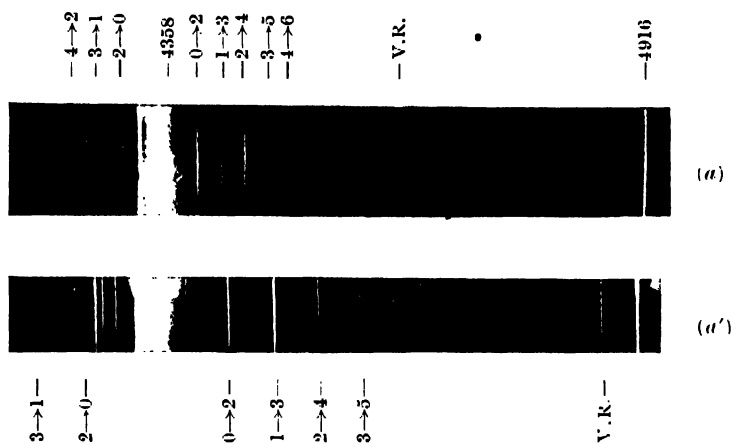
(a) Raman Spectrum of Deuterium.

(a') Raman Spectrum of Hydrogen on the same scale for comparison.

'
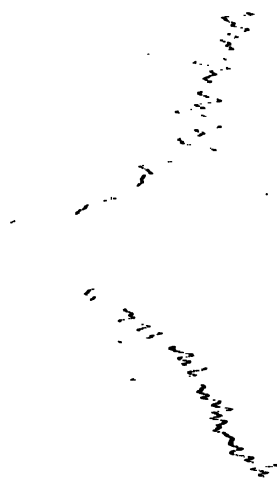
(b') Microphotometric record of the Raman Spectrum of Hydrogen on the same scale.

(b')

(b) Microphotometric record of the Raman Spectrum of Deuterium.

(b)

A more detailed and quantitative investigation of the intensity, fine structure and polarisation characters is in progress and will form the subject-matter of a further communication.

In conclusion, the author desires to express his grateful thanks to Sir C. V. Raman for his kind encouragement and a loan of the heavy water used in the present investigation.

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C. Manneback	*Z. f. Phys.,* 1930, **62**, 224; and **65**, 574.
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RAMAN SPECTRUM OF HYDROGEN DEUTERIDE.

By S. BHAGAVANTAM.

(*From the Department of Physics, Andhra University, Waltair.*)

1. Introduction.

IN an earlier paper in this Journal* the author had described the results of a study of the Raman spectrum of deuterium. Subsequent to this, a particularly intense picture of the scattered spectrum is obtained using the same sample of the gas as in the previous investigation with a view to complete the work in all its aspects. This photograph, besides showing the Raman lines of D_2 very intensely, is found to exhibit another feeble series of lines excited by λ 4358. Measurement revealed that these have their origin in the HD molecules. The extreme feebleness of the lines in comparison with the D_2 lines indicates that the HD molecules are present in a very small proportion in the sample under investigation. A search is made for the well-known Raman lines of the H_2 molecule but none has been found. It may, therefore, be concluded that the sample contains no appreciable proportion of H_2 molecules. In the present paper the results of the measurements relating to the HD series of Raman lines are given.

2. Results.

TABLE I.

Raman Spectrum of Hydrogen Deuteride.

Wave-length	Exciting line	Approx. rel. intensity	Frequency observed	Frequency calculated	Transition $J \rightarrow J'$
4479·0	4358	0	618	614·8	2 → 4
4443·9	,,	$\frac{1}{2}$	442	442·1	1 → 3
4409·6	,,	0	267	266·5	0 → 2
4308·7	,,	0	−261	,,	2 → 0

The lines having frequency shift of 442 is the strongest of the series and the corresponding line excited by λ 4046 is also seen and measured on the plate.

* See page 303 of this number.

3. Discussion of Results.

Jeppesen[1] (1934) and Beutler and Mie[2] (1934) have analysed the bands due to the HD molecule in the ultra-violet. Urey and Teal[3] (1935) have recently given the following constants for the normal state of this molecule in order to account for the experimental data of the above authors.

$$B_e = 45 \cdot 6549; \ \alpha = 1 \cdot 9928; \ \gamma = 0 \cdot 03850; \ \delta = 0 \cdot 00317; \ -D_e = 0 \cdot 02602;$$
$$\beta = 6 \cdot 58 \times 10^{-4}; \ F_e = 2 \cdot 19 \times 10^{-5}.$$

These constants are used in calculating the frequencies of the various rotation Raman lines $0 \rightarrow 2$, $1 \rightarrow 3$ and $2 \rightarrow 4$ as in the foregoing paper and the results are given in Table I for comparison. The agreement between the observed and calculated frequency shifts is very satisfactory in view of the extreme feebleness of the lines.

Another outstanding feature of the spectrum is the absence of the phenomenon of alternating intensities. The intensity rises to a maximum at the second rotation line corresponding to $1 \rightarrow 3$† and then falls off. This is in accordance with what may be expected as the molecule is composed of unlike nuclei. The relative intensities of the rotation lines that are to be expected on the basis of Manneback's expressions (Manneback,[4] 1930) are calculated and given in Table II. Same statistical weight is assigned to

TABLE II.

Calculated Relative Intensities of the Rotation Lines.

PP Series $J \rightarrow J-2$		RR Series $J \rightarrow J+2$	
Transition	Intensity	Transition	Intensity
$2 \rightarrow 0$	0·188	$0 \rightarrow 2$	0·666
$3 \rightarrow 1$	0·095	$1 \rightarrow 3$	0·786
		$2 \rightarrow 4$	0·483
		$3 \rightarrow 5$	0·176
		$4 \rightarrow 6$	0·010

1 C. R. Jeppesen, *Phys. Rev.*, 1934, **45**, 480.
2 H. Beutler and K. Mie, *Naturwiss.*, 1934, **22**, 418, and subsequent papers.
3 H. C. Urey and G. K. Teal, *Rev. Mod. Phys.*, 1935, **7**, 34.
† It may be noted that in D_2, this line is weaker than the first rotation line $0 \rightarrow 2$.
4 C. Manneback, *Z. f. Phys.*, 1930, **62**, 224; and **65**, 574.

both even and odd rotational states. The calculations are for a temperature of 30°C. and the value of B_0 is taken as $44 \cdot 67$.

The fact that the intensity reaches a maximum at the second line and then falls off is nicely confirmed. A more detailed comparison is not at present possible owing to the feebleness of the lines. The stokes lines represented by $3 \rightarrow 5$ and $4 \rightarrow 6$ and the antistokes line $3 \rightarrow 1$ have not been recorded.

In conclusion the author desires to express his grateful thanks to Sir C. V. Raman for his kind interest in the work.

4. Summary.

Using $\lambda 4358$ as the exciting radiation, frequency shifts of 267, 442 and 618 arising respectively from the rotational transitions $0 \rightarrow 2$, $1 \rightarrow 3$ and $2 \rightarrow 4$ have been recorded in the Raman spectrum of hydrogen deuteride gas. The figures compare well with $266 \cdot 5$, $442 \cdot 1$ and $614 \cdot 8$ which are calculated on the basis of the molecular constants given by Urey and Teal for the HD molecule. The antistokes line arising from the transition $2 \rightarrow 0$ is also recorded. The phenomenon of alternating intensities is not observed and the line corresponding to $1 \rightarrow 3$ is the most intense one in the series as may be expected.

HYPERFINE STRUCTURE IN SELENIUM, PALLADIUM AND GOLD.*

By L. Sibaiya.

(*From the Department of Physics, Central College, Bangalore.*)

Selenium (At. no. 34 ; At. wt. 79·2).

For purposes of classification of selenium spectra, the arc and spark lines of selenium have been mostly excited in tubes of various types with condensed or uncondensed electrical discharge through selenium vapour or between aluminium poles tipped with metallic selenium. A selenium arc either in vacuum or in an atmosphere of nitrogen has also been employed. Such sources however are not suited for hyperfine structure work, as the lines obtained are broad and diffuse. Moreover most of the prominent arc lines of selenium lie either in the near infra-red or in the extreme ultra-violet, thus rendering their analysis by high resolving power apparatus specially difficult. Some intense spark lines of selenium lie in the visible region ; but under the conditions employed for their emission in discharge tubes, the broadening of the lines renders such sources unsuitable for hyperfine structure study. The apparatus used, the essential part of which is a water-cooled hollow cathode, is the same as that employed by Prof. Venkatesachar and the author in their investigation on the isotopic constitution of platinum.[1] The selenium powder took the place of the platinum foil in the tubular space of the cathode (Pl. XV, Fig. 1). For experimental details the above paper must be consulted.

Of the seventeen lines of selenium here examined, only two are arc lines and the remaining fifteen belong to the first spark spectrum of selenium.

Se I LINES.

λ in Å.U.	Classification
4739·03	$5s(^4S)$ $^5S_2$—$6p(^1S)$ $^5P_2$
4730·79	$5s(^4S)$ $^5S_2$—$6p(^1S)$ $^5P_3$

The spectral classification of the arc lines is taken from Meissner, Bartelt and Eckstein.[2]

The spark lines here analysed are listed below ; they have been classified differently by Bartelt[3] and Krishnamurthy and Rao[4]:

<center>Se II LINES (λ in Å.U.).</center>

5305·41	5143·15	4840·61
5253·69	5068·67	4763·66
5253·13	4992·88	4648·40
5227·53	4975·76	4618·75
5175·97	4844·98	4602·32

All the above Se I and Se II lines are sharp and single (Pl. XV, Fig. 2). Selenium, according to Aston, has the following isotopic constitution :

Mass number			74	76	77	78	80	82
Percentage abundance			0·9	9·5	8·3	24·0	48·0	9·3

The even isotopes of selenium amount to 91·7% ; the single odd isotope Se 77 is present only to the extent of 8·3%. It is therefore difficult to observe the components arising from a splitting of the gross multiplet levels due to a nuclear spin in Se 77. In each of the lines examined the observed component is to be ascribed to the even isotopes whose total abundance is 91·7%. Even isotope displacement, if any, could have been detected because Se 80 (48%) is twice as abundant as Se 78 (24%), the other two Se 76 and Se 82 being nearly equally abundant (9·4%). The absence of structure revealed by the lines indicates that none of the levels here observed shows any even isotope displacement and that all the even isotopes of selenium have nuclear spins equal to 0. From an examination of two lines in the arc spectrum of selenium Rafalowski[5] has come to the same conclusion. Though the absence of structure leads to the conclusion that the nuclear magnetic moment of Se 77 is small, there is however a faint component (~ +0·093 cm.[-1] ?) that accompanies 5227·53 Å, a fact which can be most simply explained by ascribing a nuclear spin of $\frac{1}{2} \frac{h}{2\pi}$ to the odd isotope 77.

[2] Meissner, etc., *Zeits. f. Physik*, 1934, **91**, 432.
[3] Bartelt, *Zeits. f. Physik*, 1934, **91**, 450.
[4] Krishnamurthy and Rao, *Proc. Roy. Soc.*, 1935, **149**, 56.
[5] Rafalowski, *Acta Physica Polonica*, 1933, **2**, 119.

Palladium (At. no. 46 ; At. wt. 106·7).

Since the isotopic constitution of palladium by the mass-spectrograph method has not been found,† the hyperfine structure of the palladium lines is here studied with the object of determining, if possible, the isotopes of palladium. A thin palladium foil‡ is introduced into the hollow cathode, which is excited by a D. C. 1 kilowatt generator with a discharge current of 200 mA at 1700 v. The following arc lines were analysed ; the classification of the lines is taken from Shenstone[6]:

Pd I LINES.

λ in Å.U.	Classification	
3242·72	$5s\ ^3D_3$—$5p\ ^3D_3^{o}$ •	
3251·66	$5s\ ^3D_1$—$5p\ ^1P_1^{o}$	
3302·15	$5s\ ^3D_1$—$5p\ ^3D_1^{o}$ •	
3404·60	$5s\ ^3D_3$—$5p\ ^3F_4^{o}$ •	
3421·24	$5s\ ^3D_2$—$5p\ ^3D_2^{o}$	
3433·41	$5s\ ^1D_2$—$5p\ ^1P_1^{o}$	
3441·10	$5s\ ^1D_2$—$5p\ ^1D_2^{o}$	*Vide* Pl. XVI, Fig. 3.
3460·76	$5s\ ^3D_3$—$5p\ ^3F_3^{o}$	
3481·17	$5s\ ^3D_1$—$5p\ ^3F_2^{o}$ •	
3489·79	$5s\ ^1D_2$—$5p\ ^3D_1^{o}$	
3516·95	$5s\ ^3D_2$—$5p\ ^3P_1^{o}$	
3553·10	$5s\ ^1D_2$—$5p\ ^1F_3^{o}$	
3609·56	$5s\ ^3D_2$—$5p\ ^3F_3^{o}$	
3634·70	$5s\ ^3D_3$—$5p\ ^3P_2^{o}$	

None of the lines above shows any isotope displacement of the even isotopes. It has therefore not been possible to determine the even isotopes of palladium or their relative abundance. A study of the known isotopes of elements in the neighbourhood of palladium shows that an odd isotope of mass number 105 should be expected to exist in palladium. Though most of the lines here examined are single, the existence of a close component ($\sim +0 \cdot 100$ cm.$^{-1}$) in a few lines renders it probable that the isotope Pd 105 with an abundance of the order of 15% has a nuclear spin $\frac{1}{2}\frac{h}{2\pi}$. The general absence of structure however indicates that the nuclear magnetic moment of Pd 105 is small. These observations in selenium and palladium support the conclusion that all nuclei with even atomic number and odd mass number have only small positive or negative magnetic moments.[7]

Gold (At. no. 79 ; At. wt. 197·2).

Frisch[8] has concluded that the resonance line of gold $\lambda\,2676$ Å ($6^2S_{\frac{1}{2}}$— $6^2P_{\frac{1}{2}}$) is single ; Ritschl[9] finds on the other hand that the two resonance lines $\lambda\,2676$ Å and 2428 Å ($6^2S_{\frac{1}{2}}$—$6^2P_{\frac{1}{2},\frac{3}{2}}$) are each double. The satellites are observed at $+0\cdot224$ cm.$^{-1}$ and at $+0\cdot221$ cm.$^{-1}$ in the two lines respectively. The existence of this structure has been attributed by Ritschl to a nuclear spin of $\frac{3}{2}\frac{h}{2\pi}$. Since the two above lines are resonance lines, self-absorption in the source by normal gold atoms can produce a doubling of each of the resonance lines. The present work has been undertaken with the object of deciding between these conflicting results ; it has been definitely shown that Ritschl's analysis of the resonance lines is correct. Wulff[10] has reported that his results agree with those of Ritschl ; but it has been re-marked that a nuclear spin value of $\frac{5}{2}$ satisfies the interval rule better in a number of levels. In the present work the lines involving levels known to show isotope shift in the isoelectronic Hg II spectrum[11] are analysed, and it is concluded that gold consists of a single isotope of mass 197.

A hollow cathode made from a sheet of copper-gold alloy (containing about $0\cdot5\%$ gold) was first employed. $\lambda\,2676$ Å exhibited two components of nearly equal intensity ; the possibility of this doubling arising out of self-reversal could not be ruled out. Hence the water-cooled hollow cathode previously described[1] was gold-plated on the inside ; an examination of $\lambda\,2676$ Å revealed again the same two components. If the observed doublet

[7] Grace, *Phys. Rev.*, 1933, **44**, 362.
[8] Frisch, *Zeits. f. Physik*, 1931, **71**, 92.
[9] Ritschl, *Naturwiss.*, 1931, **19**, 690.
[10] Wulff, *Phys. Rev.*, 1933, **44**, 512.
[11] Venkatesachar and Sibaiya, *Proc. Ind. Acad. Sci.*, 1934, **1**, 8.

structure is not caused by any reversal, it was argued that under suitable conditions of excitation each of these components would be reversed and four lines could be observed. A thin gold sheet was therefore introduced into the hollow cathode and a discharge current of 200 mA at 1000 v. from a D. C. 1 kilowatt generator was maintained. Pl. XVI, Fig. 4 shows the doubling of each of the two real components of $\lambda\,2676$ Å due to self-reversal in the source. Thus it has been established that $\lambda\,2676$ Å of gold consists of two components, *viz.*, $0 \cdot 000$ and $+0 \cdot 223$ cm.$^{-1}$, with an intensity ratio of about $3:2$. The satellite separation has been computed from all the above methods, including the one giving self-reversed components.[12]

The nuclear spin of gold has to be deduced from the intensity ratio of the components; it is however difficult from visual estimates of intensities to decide between the nuclear spin values $\frac{3}{2}$ and $\frac{5}{2}$, which demand the intensity ratios to be $1 \cdot 67$ and $1 \cdot 40$ respectively.* The balance of evidence is in favour of the value $\frac{3}{2}$ because the following lines involving the meta-stable level $5d^9\,6s^2\,{}^2D_{\frac{5}{2}}$ have the appearance of incompletely resolved flag patterns containing four components; nuclear spin values of $\frac{3}{2}$ and $\frac{5}{2}$ should yield four and six components respectively.

λ in Å.U.	Classification	Total width in cm.$^{-1}$	Interval factor of ${}^2D_{\frac{5}{2}}$
$3029 \cdot 22$	$5d^9\,6s^2\,{}^2D_{\frac{5}{2}}$—$5d^9\,6s6p\,{}^2F_{\frac{7}{2}}$	$0 \cdot 163$	$0 \cdot 018$ cm.$^{-1}$
$2748 \cdot 26$	$5d^9\,6s^2\,{}^2D_{\frac{5}{2}}$—$5d^9\,6s6p\,{}^4F_{\frac{7}{2}}$	$0 \cdot 165$	

The hyperfine levels in ${}^2D_{\frac{5}{2}}$ are inverted.

One would expect that if gold should consist of two isotopes 197 and 199 as is suggested from its chemical atomic weight, the above lines should show the structure patterns due to the two isotopes separately. This is necessary because the $5d^9\,6s^2\,{}^2D_{\frac{5}{2}}$ level in the isoelectronic spectrum of Hg II shows large isotopic displacement. The photographs of the hyperfine structure patterns of $\lambda\,3029$ Å and 2748 Å are so well exposed that even if Au 199 should exist to the extent of about 5% its presence could not have escaped notice. It must be concluded that gold consists of a single isotope of mass 197 and that its accepted chemical atomic weight is too high. Further support to this conclusion is given by the fact that an odd isotope of an element with odd atomic number has no isobare in appreciable quantity;

[12] Sibaiya, *Proc. Ind. Acad. Sci.*, 1934, 1, 321.

Au 199, if it should exist, would be an isobare with Hg 199 (16·45%). The following table gives the percentage abundance of isobares with odd mass numbers in the heavy elements.

At. No.	Element	Percentage abundance of odd isobares				
75	Re	187 (61·8%)				
76	Os	187 (0·6%)				
79	Au		197 (100%)			
80	Hg		197 (0·01%)	203(0·006%)		
81	Tl			203 (29·1%)	205 (70·6%)	
82	Pb			203 (0·01%)	205 (0·03%)	209 (0·85%)
83	Bi					209 (100%)

Thus Au 199, if it does exist, must be present to an extent not exceeding 1% and probably it is entirely absent,* because whenever an element with odd atomic number contains two odd isotopes they will exist in comparable quantities.

The nuclear $g(I)$ factor can be computed from Goudsmit's formula for a penetrating s-electron[13] :

$$g(I) = \frac{3a}{8R\alpha^2} \times \frac{n_0^3}{Z_i Z_n^2} \times \frac{1838}{K(j, Z_i)}.$$

For the normal $6s\ ^2S_{\frac{1}{2}}$ state of gold $a = -0·112$ cm.$^{-1}$, $n_0 = 1·214$ and K $(j, Z_i) = 2·2$; and the $g(I)$ value becomes $0·136$ while the magnetic moment of the nucleus is $0·20$. White[11] gives the nuclear magnetic moment as $1·8$ after Fermi and Segre[15] ; Schüler[16] however obtains the value $0·15$ for the $g(I)$ factor. My calculated value of $0·136$ for the $g(I)$ factor agrees well with the theoretical value $0·133$ of Lande.[17] Using Goudsmit's formulæ for the

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* More recently, Dempster (*Nature*, 1935, **136**, 65) has obtained the same result with the aid of his mass-spectrograph.

[13] Goudsmit, *Phys. Rev.*, 1933, **43**, 636.

[14] White, *Introduction to Atomic Spectra*, 1934, p. 372.

[15] Fermi and Segre, *Zeits. f. Physik*, 1933, **82**, 729.

[16] Schüler, *Zeits. f. Physik*, 1934, **88**, 323.

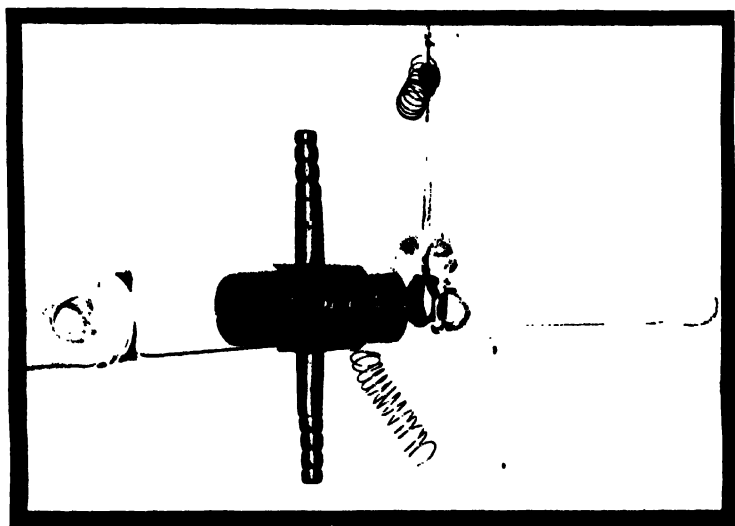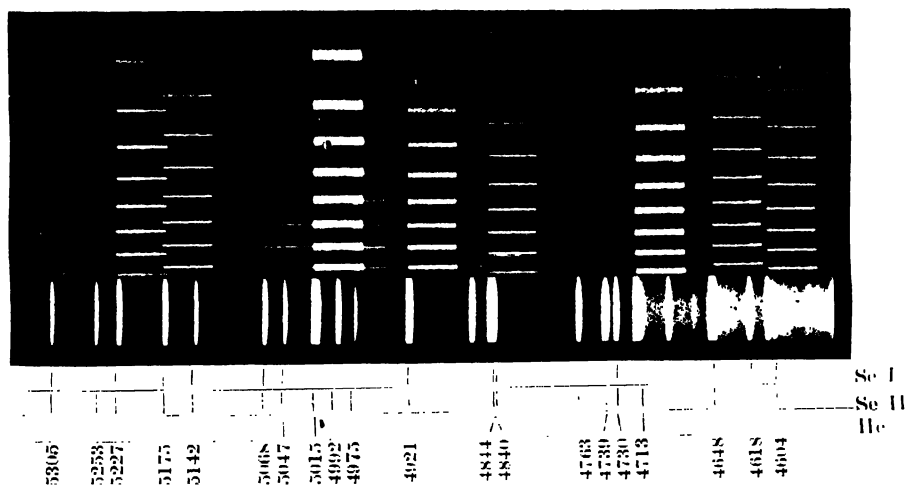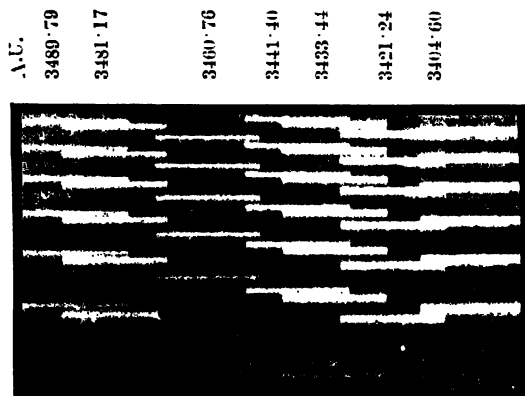[17] Lande, *Phys. Rev.*, 1934, **46**, 477.

FIG. 1.
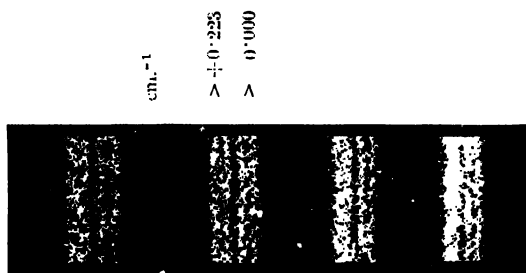

FIG. 2.

FIG. 3. Structure Pattern of Palladium Arc Lines.

FIG. 4. Structure Pattern of Au I 2676A ($6\,^2S_{1_2}$ $6\,^2P_{1_2}$)
with each of the two components doubled by
self-absorption in the source.

p-electrons it is found that the levels $5d^{10}\,6p\ ^2P\frac{1}{2},\frac{3}{2}$ have total separations amounting to $0 \cdot 024$ cm.$^{-1}$ and $0 \cdot 008$ cm.$^{-1}$ respectively. These separations are too small to be resolved in the lines involving these levels. The larger separation ($\sim 0 \cdot 164$ cm.$^{-1}$) with an interval factor of $0 \cdot 018$ cm.$^{-1}$ in $5d^9\,6s^2$ $^2D\frac{5}{2}$ is consistent with theoretical expectations.

In conclusion I wish to thank Prof. B. Venkatesachar for his helpful guidance.

Summary.

Hyperfine structure analysis of some selenium and palladium lines shows that none of the levels examined reveal any even isotope displacement. The nuclei of Se 77 and Pd 105 have very small magnetic moments and their spin moment is probably $\frac{1}{2}\,\dfrac{h}{2\pi}$.

The doublet structure ($\Delta\nu - 0 \cdot 224$ cm.$^{-1}$) observed by Ritschl in the resonance lines of gold has been confirmed by the redoubling of each component due to self-reversal in the source; this test proves that the originally observed doublet structure does not arise from self-reversal as the earlier results of Frisch would suggest. While in the isoelectronic spectrum of Hg II the $5d^9\,6s^2\,^2D\frac{5}{2}$ level exhibits isotope displacement, arc lines of gold involving this level point definitely to the existence of a single isotope of mass 197; the accepted chemical atomic weight is therefore considered to be too high. The nuclear spin moment of gold is $\frac{3}{2}\,\dfrac{h}{2\pi}$ and the $g(I)$ factor comes out as $0 \cdot 136$ agreeing with Lande's theoretical value.

PROCEEDINGS

OF

THE INDIAN ACADEMY OF SCIENCES

CONTENTS

SECTION B

PAGE

THE USE OF ACTIVATED CARBON IN THE PURIFICATION OF WATER IN THE TROPICS. (THE MADRAS CITY WATER SUPPLY.)

By T. N. S. RAGHAVACHARI, B.A.,

AND

P. V. SEETHARAMA IYER, M.A.

(From the King Institute of Preventive Medicine, Guindy.)

Introductory.

THE use of wood-charcoal and bone-char for clarifying and removing colour from liquids and for adsorbing noxious gases is well known. Vegetable carbon came into extensive use during the Great World War as one of the most powerful adsorbing agents for use in gas-masks, as it was found to possess greater adsorbing power than bone-char.

The first suggestion to use activated vegetable carbon for purifying waters was made by Sauer of the Algemeene Norit Maatschappij of Amsterdam in 1920 (Liddle, 1931, 1932). In 1928, Imhoff and Sierp of the Ruhr Valley Union succeeded in installing an activated carbon filter at Haam capable of producing 5½ to 7 million gallons of good potable water every day. Watzl (1929) carried this idea into America but did not succeed in introducing it there. Baylis (1929, 1930 *a*, 1930 *b*, 1931, 1932 1932 *b*) carried out an elaborate study of this material and proved that activated carbon was a powerful agent for removing tastes and odours from drinking water. Activated carbon filters are reported to be functioning well in several cities in Holland and Germany. There is one such plant in effective use at the water works of the South End Water Co., in England. Sir Alexander Houston (1930, 1931), has also reported very favourably on its use. In America, at the present day, there are over 750 plants that use activated carbon for the removal of tastes and odours. The majority of these plants use powdered activated carbon, but activated granular carbon filters are in successful use at Chicago and Dundee, as also in Bay City.

During the comparatively short time that activated carbon has been in use in water purification processes, a very large volume of literature has accumulated on the value of the material for removing tastes and odours in water supplies. We have carefully perused nearly all the published

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literature on the subject and we give below the more important findings gleaned in our study.

1. Tastes and odours caused by chlorine, phenols, chlorophenols and related compounds are readily removed by treatment with activated carbon.

2. Tastes and odours caused by decomposing algal and vegetable growths are also similarly removed.

3. The great value of activated carbon in the treatment of drinking water lies in its wonderful powers of adsorption and its remarkable ability to remove colloids from water.

4. It is, therefore, able to effect the *removal* of tastes and odours in contrast to the *prevention* of the same.

5. When added with alum to a water, as in the treatment of water through mechanical rapid filters, activated carbon greatly assists floc-formation and thereby materially reduces the dose of alum.

6. There is practically no taste or odour likely to occur in water supplies which cannot be removed by the use of activated carbon (Thresh *et al*, 1933).

7. It removes colour, free chlorine and metallic impurities, such as lead, iron and manganese.

Activated Carbon.

Mantell (1928, 1931) describes at length the process of manufacture and the varied uses to which activated carbon may be put. The raw materials from which activated carbon suitable for water purification is ordinarily manufactured are lignite, waste liquors, oil residues, saw dust, cocoanut and other shells and nearly all kinds of wood. These are carbonised in closed retorts under controlled conditions of temperature and pressure. The product is an amorphous carbon of optimum density free from stabilised and adsorbed hydrocarbons. Steam activation is better than air activation and $900°$ C. to $950°$ C. is the most suitable temperature.

The excellent results reported in the published literature attending the use of activated carbon for the removal of tastes and odours in water prompted us to investigate the possibilities of applying it to the Madras water supply. The city of Madras derives its supply of drinking water from the Red Hills lake. This lake has an extensive catchment area of its own and is also fed by another lake which receives its supply from the Courtelliar river. This river drains 940 sq. miles of grazing and inhabited land. The organic pollution thus brought into the Red Hills lake is very great. It increases with storage—a result which is largely due to the growth and subsequent decay of algal forms of life. King, Raghavachari and Narasimha Iyengar (1930) showed that 80 per cent. of the organic matter in the lake water was

not centrifugeable and so was present probably in colloidal form. The average Tidy's figure for organic matter in the lake water is 0·180 parts per 100,000. As a result of the growth and decay of algal life there is a great depletion of the dissolved oxygen in the lower layers of the lake. The average deficiency in oxygen saturation in the lake water is about 26 per cent. (Raghavachari and Ganapathi, 1929). This is not large. The point about the Red Hills lake water is not the oxidisable organic matter, nor the absolute deficiency of the dissolved oxygen, but the deficiency of the latter relative to the excessive amount of oxidisable organic matter carried.

Slow sand filters were installed at Kilpauk in 1914, for purifying the Red Hills lake water. During the twenty years that they have been in existence, they have not produced potable water of uniform quality. Tastes and odours have always been present in the filtered supply. Sulphuretted hydrogen is produced in the filters in large quantities and post-filtration growths of filamentous and other low forms of life occur in the underground tanks and in the pipe-lines. The production of sulphuretted hydrogen in the filters has been ascribed to the anærobic conditions and putrefactive processes set up in the underlayers of the filters.* Dey and Ganapathi (1934) have adduced some evidence—not convincing enough however, to us—that the production of sulphuretted hydrogen in the Madras filters is almost entirely due to the reduction of the sulphates present in the water by the *Spirillum desulphuricans*. Whether the gas is derived immediately from the sulphate-reduction, or from the excessive organic matter, it is still true, we think, that the anærobic condition prevailing in the lower layers of the filters due to the relatively large excess of colloidal organic matter and the poor content of dissolved oxygen in those layers, accounts in a large measure for the production of sulphuretted hydrogen. A reduction in the organic content and an increase in the dissolved oxygen content of the water as distributed should, therefore, be the aim of any measure designed for the improvement of the City water-supply.

Our experience with activated carbon for achieving this end will, therefore, now be described under two separate headings :—

I. Experiments on the use of (*a*) powdered activated carbon and (*b*) granular activated carbon, on a small plant scale for the removal of the colour, taste and odour found in the filtered (12″ v.p.h.) chlorinated water supplied to the City.

II. Experiments on the use of granular activated carbon (i) as an auxiliary medium in a slow sand filter for removing any earthy or musty

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* *Reports of the Government Committee on Water and Sewage Purification*, 1920 to 1932.

taste present in the applied raw water and for preventing, at the same time, the formation of odours in the slow sand filter (*e.g.*, H_2S, etc.) and (ii) as a contact bed for the removal of colour, taste and odour formed in the process of filtration through a normal slow sand filter working at 4" vertical per hour.

I. *Experiments on Powdered and Granular Carbon.*

In the summer of 1932, a local English firm of aerated water manufacturers experienced enormous difficulties in providing a taste and odour-free product to their customers, using the municipal supply, which developed a *very marked* taste and odour, such as had not been experienced in previous years. The usual practice at this factory was to pass the city supply through two units (8' × 3' × 6' each) of sand beds—3 feet of coarse sand alone was used as filtering medium—and then to lead the effluent from these beds through two batteries of 42 Berkefield candles each and finally to treat the double-filtered water with ultra-violet light. In normal years, this system was found to work fairly well and to give them a water which was very much better than the indifferent City supply. The troubles met with in 1932, however, made the firm realise the urgent necessity for devising and introducing suitable safeguards in their plant against a recurrence of the trouble in future years. The opportunity was taken by the senior author to make use of the desire and willingness of this firm to permit him to conduct, at their cost, experiments on taste and odour removal by using activated carbon.

(*a*) *Powdered Carbon.*—This firm suggested that he tried the brand of activated carbon* for which they had obtained quotations. Accordingly, a supply of this in powder form was obtained about the end of January 1933, and experiments started. The coarse sand in the filter permitted the free passage of the applied carbon powder, through its entire depth; an 18" layer of fine sand (passing through 10 and retained on 40 meshes to the sq. inch) was therefore laid on top of the coarse sand in both the units. These units functioned only intermittently, *i.e.*, worked between 8 A.M. and 5 P.M. on week days. Two ounces by weight of powdered active carbon—3 p.p.m.—were sprinkled once a week on Mondays, on the surface of the water in one of the units, the other being worked as a control. The results of two sets of experiments on these lines conducted between 7–2–1933 and 12–5 1933 will be found in Tables I and II.

Note:—For convenience in comparison, we have adopted an arbitrary scheme of assessing certain of the more important results by giving numerical values to grade them. Unity represents in every case the maximum purity attainable and numbers higher than unity indicate a progressive order of

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* Jewlcarbon, of Messrs. The Jewell Export Filter Co., Calcutta.

demerit. This scheme, designed by Lieut.-Colonel H. H. King, C.I.E., I.M.S., in 1929 has since been adopted by us at this Institute in our special research reports. It will be reproduced here for reference.

Items that have been graded (arbitrarily)—

(*a*) *Colour-Grade.*—(1) Colourless, (2) Slightly yellowish, (3) Yellow, (4) Muddy or brown.

(*b*) *Clarity-Grade.* (1) Clear, (2) Slightly hazy, (3) Hazy, (4) Opaque.

(*c*) *Lactose Fermenters.* Grade (1) Absent in 60 c.c., (2) Present in 60 c.c., (3) Present in 20 c.c., (4) Present in 10 c.c., (5) Present in 5 c.c.

(*d*) *Taste and Smell.* Results of tests made each time by different intelligent, independent observers (one or two being constant, the rest being variable). (1) Absent, (2) Vague and just noticeable, (3) Pronounced earthy or musty, (4) Disagreeable, medicinal.

(*e*) *Presence of Visible Suspended Matter.*—(Sample 500 c.c. drawn in a glass tumbler and observed against transmitted light.) $+ +$ or $+$ Present. $+$ Slight. $-$ Absent.

(*f*) The figures for albuminoid and free ammonias and for absorbed oxygen (Tidy's) in 4 hours represent parts per 100,000.

(*g*) The figure for dissolved oxygen is in c.c. per litre.

TABLE I.

Period of test : 7-2-33 to 23-2-33. *Results (average of 7 tests).*

	Feed Water from City Mains	Carbon Unit	Control
Total count per c.c. on agar at 37° C.	250	300	380
Lactose fermenters (grade)	2·2	3·2	3·5
Colour (do.)	2·4	1·0	2·4
Clarity (do)	1·1	1·0	1·1
Taste and smell (do.)	3·0	1·0	3·0
Suspended matter	$+$	$-$	$-$
Free ammonia (parts per 100,000)	0·002	0·001	0·002
Albuminoid ammonia (do.)	0·021	0·015	0·019
Oxygen absorbed (do.)	0·086	0·066	0·082
Dissolved oxygen (c.c. per litre)	2·2	4·6	1·8
pH (colorimetric)	7·5	7·5	7·5

TABLE II.

Period of test : 3-3-33 to 12-5-33. *Results* (*average of* 18 *tests*).

	Feed Water from City Mains	Carbon Unit	Control
Total count per c.c. at 37° C. ..	260	100	450
Lactose fermenters (grade) ..	2·3	1·2	3·7
Colour (do.) ..	2·8	1·0	1·7
Clarity (do.) ..	1·5	1·0	1·2
Taste and smell (do:) ..	3·0	1·0	2·0
Suspended matter ..	+	—	±
Free ammonia (parts per 100,000)	0·004	0·001	0·001
Albuminoid ammonia (do.) ..	0·017	0·012	0·014
Oxygen absorbed (do.) ..	0·099	0·066	0·080
Dissolved oxygen (c.c. per litre) ..	2·5	4·7	4·0
pH ..	7·5	7·5	7·6

The results of these preliminary tests showed that the use of powdered activated carbon effected great improvement in the æsthetic qualities of the water and at the same time brought about a noticeable reduction in the organic and bacterial content of the water as compared with the control.

It became necessary to add powdered carbon to both the units as the firm could ill-afford to run a control indefinitely, when taste and odour troubles were becoming accentuated in the summer months.

In the next experiment from 20-6-1933 to 5-9-1933, powdered active carbon was added every day at the rate of ¼ oz. to each of the two units by the sprinkling method. The dose worked out to between 3 and 3·5 p.p.m. The results of this experiment in Table III were confirmatory of the previous findings.

In May and again in September 1933, when the beds using carbon were stopped arbitrarily for cleaning, it was found that the carbon powder had

TABLE III.

Period of test : 20-6-33. *Results (average of 20 tests).*

	Feed Water from City Mains	P.C. 1 Carbon Unit Old	P.C. 2 Carbon Unit New
Total count on agar at 37° C.	420	60	130
Lactose fermenters (grade)	2·7	1·3	2·0
Colour (do.)	2·8	1·0	1·0
Clarity (do.)	1·0	1·0	1·0
Taste and smell (do.)	3·5	1·0	1·0
Suspended matter	+		
Free ammonia (parts per 100,000)	0·005	0·002	0·002
Albuminoid ammonia (do.)	0·022	0·012	0·013
Oxygen absorbed (do.)	0·118	0·080	0·091
Dissolved oxygen (c.c. per litre)	2·1	4·8	4·6
pH	7·6	7·5	7·5

penetrated down only to within an inch and a half of the top fine sand layer, but our attempts to recover the carbon from the above layer were unsuccessful. We concluded that the use of powdered carbon would therefore be less economical in the long run than granular carbon, if the latter, which was reputed to last for 3 to 5 years, could be relied upon to yield equally good results.

(b) *Granular "Jewlcarbon".* - Accordingly a comparison was made using powdered carbon on one bed and granular carbon on the other. Smit (1933) in Alblaserdam, found a thin layer of granular carbon 1½" thick, and sandwiched between two layers of sand in a sand filter to give very good results. It was decided to copy that plan in one of the units. A thin layer of granular carbon 1" thick was sandwiched between 6" and 12" of fine sand in one of the two units at the factory and the other unit was fed with the powder as in the previous experiments. The results obtained will be found in Table IV.

TABLE IV.

Period of test: 14-9-33 *to* 4-10-33.   *Results*   (*average of* 8 *tests*).

	Feed Water from City Mains	P.C. 2 Powder Carbon	G.C. 1 Granular
Total count on agar at 37° C.	100	70	110
Lactose fermenters (grade)	2·4	1·9	2·0
Colour (do.)	2·8	1·0	1·0
Clarity (do.)	1·2	1·0	1·0
Taste and smell (do.)	3·0	1·0	1·0
Suspended matter	++	...	--
Free ammonia (parts per 100,000)	0·004	0·002	0·001
Albuminoid ammonia (do.)	0·030	0·016	0·012
Oxygen absorbed (do.)	0·157	0·080	0·060
Dissolved oxygen (c.c. per litre)	1·9	4·4	4·8
pH	7·8	7·8	7·6

These results show conclusively that the granular carbon bed yielded a filtrate which was superior to the powder-fed filter in every respect. The added advantage of not having to mess about with the powder every day, lent weight to our decision to use granular carbon in both the units. The second unit was accordingly relaid after a thorough cleaning, and the final experiment carried out between October 1933 and March 1934. The results given in Table V show that the granular carbon in the manner used by us, is indeed quite as efficient as, if not more than, the powder in removing colour, taste and odour, as also in effecting a general all-round improvement in the water as supplied to the City.

These two units have been functioning well up to the present (August 1935). They have not been subjected to any special treatment, other than the ordinary cleaning and relaying of the top layers of 6″ of sand and 1″ of carbon, twice during the entire period.

PART II.—*Experiments at Kilpauk Waterworks, under the direction of the Sanitary Engineer to Government and the Director, King Institute. These*

TABLE V.

Period of test : 17-10-33 *to* 15-3-34. *Results* (*average of 30 tests*).

	Feed Water from City Mains	G.C. 1 Granular Bed Old	G.C. 2 Granular Bed New
Total count on agar at 37° C.	120	55	80
Lactose fermenters (grade)	1·8	1·2	1·8
Colour (do.)	2·8	1·0	1·0
Clarity (do.)	1·4	1·0	1·0
Taste and smell (do.)	2·0	1·0	1·0
Suspended matter	++	—	—
Free ammonia (parts per 100,000)	0·006	0·001	0·001
Albuminoid ammonia (do.)	0·027	0·012	0·011
Oxygen absorbed (do.)	0·123	0·050	0·044
Dissolved oxygen (c.c. per litre)	3·8	4·8	4·9
pH	7·4	7·4	7·4

experiments were done at the Experimental Filter Plant maintained under the Madras Government.

(i) Granular carbon in a thin layer (1½″) sandwiched between the fine and coarse sand layers in a slow sand filter.

Preliminary experiments on a laboratory scale were conducted using glass tubes 4½′ long and 1½″ diameter, filled with filtering material in the following order from the top ; 18″ fine sand, supported on 6″ of coarse sand in the control, and 18″ fine sand supported on a 4½″ layer of granular carbon in the other tube. This preliminary experiment lasted from 1-6-33 to 16-8-33. The results afforded evidence of the superiority of the carbon unit as compared with the control in dealing with the raw water of the Red Hills lake, which was fed to both.

It was decided, therefore, to repeat this experiment on a small plant scale. One of the two slow sand filters received a sandwiched layer of granular carbon 1½″ thick, while the other served as a control. Both the units ran at 4″ vertical per hour continuously, between 15-10-33 and 30-6-34. The results are given in Table VI (cold weather) and Table VII (hot weather).

TABLE VI.

Period (i): 15-10-33 *to* 28-2-34.   *Cold Weather Conditions.*

Results (average of over 50 tests).

	Raw Water	Control Slow Sand Filter at 4″ v.p.h.	Carbon Sandwiched Sand Filter at 4″ v.p.h.
Total count per c.c.	1300	190	200
Lactose fermenters (grade)	5·1	2·9	2·6
Colour (do.)	3·0	2·8	1·5
Clarity (do.)	2·8	1·4	1·0
Taste and smell (do.)	2·0	3·6	1·4
Free ammonia (parts per 100,000)	0·027	0·018	0·016
Albuminoid ammonia (do.)	0·027	0·018	0·016
Oxygen absorbed (do.)	0·141	0·139	0·077

TABLE VII.

Period (ii): 1-3-34 *to* 15-8-34.   *Hot Weather Conditions.*

Results (average of about 50 tests).   *(a)* 1–3–34 *to* 30- 6 -34.

	Raw Water	Control Filter t 4″ v.p.h.	Carbon Sandwiched Sand Filter at 4″ v.p.h.
Total count per c.c. ..	1600	250	160
Lactose fermenters (grade) ..	5·1	1·6	1·9
Colour (do.) ..	3·0	2·8	1·6
Clarity (do.) ..	1·4	1·1	1·0
Taste and smell (do.) ..	3·0	4·0	1·9
Free ammonia (parts per 100,000)	0·002	0·014	0·011
Albuminoid ammonia (do.) ..	0·029	0·015	0·020
Oxygen absorbed (do.)	0·161	0·231	0·088

The carbon unit yielded definitely better results than the control during both periods. Sulphuretted hydrogen was produced, as usual, in large quantities in the control filter but only in minute traces, in the carbon unit. The effluent from the latter was otherwise better than the control.

Our experience with the Madras water has been that the slow rate of 4″ vertical per hour is conducive to the production of H_2S, while at higher rates (of say, 8″ and 12″ v.p.h.) H_2S was produced rarely, if at all. It was, therefore, decided to run the carbon unit at a higher rate to see if the traces of H_2S and the consequent slight taste and odour in the effluent could be eliminated. The rate was therefore increased to 8″ vertical per hour in the carbon unit only, from the 1st July 1934. The results obtained from that date to the 15th August 1934 (Table VIII) show that sulphuretted hydrogen was not produced even in traces, and that the effluent was free from taste or smell. There was also a greater reduction in the organic content of the effluent from the carbon unit.

TABLE VIII.

Period of test : 1-7-34 *to* 15-8-34. *When the* carbon filter *was* worked *at a semi-rapid rate of* 8″ *v.p.h.*

	Raw Water	Control Filter at 4″ v.p.h.	Carbon Sandwiched Sand Filter at 8″ v.p.h.
Total count per c.c. ..	1000	500	300
Lactose fermenters (grade) ..	5·7	4·2	4·2
Colour (do.) ..	3·0	2·8	1·2
Clarity (do.) ..	1·5	1·1	1·0
Taste and smell (do.) ..	3·0	4·0	1·0
Free ammonia (parts per 100,000)	0·001	0·010	0·001
Albuminoid ammonia (do.) ..	0·047	0·024	0·023
Oxygen absorbed (do.) ..	0·191	0·171	0·087

In the above experiment, a thin layer of granular carbon in a submerged location was expected to deal effectively with a raw water, rich in colloidal organic matter and to prevent the formation of tastes and odours ; activated granular carbon is generally used in deep contact beds for the removal of *tastes and odours already formed* ; *i.e.*, for the *removal* and *not prevention* of

tastes and odours. In the latest edition of *The Examination of Water and Water Supplies* by Thresh, Beale and Suckling (1933), this view is favoured and advice given against the inclusion of granular carbon in a sand filter. Our last experiment conducted during the worst period of the year has, however, proved that the granular carbon, even in the sandwiched position, is capable of functioning under certain conditions.

Further experiments with different thicknesses, placed at different situations in a sand bed would appear to be necessary (the rate of filtration not being lower than 8" v.p.h.) before discarding this method of using granular carbon.

(ii) The use of activated granular carbon—as such—in a contact bed for taste and odour removal.

A small contact filter ,made of a piece of stoneware pipe 2' high and 9" diameter was filled with 1½' of granular " Jewlcarbon " and the effluent from our control slow sand filter containing large and varying quantities of H_2S and filamentous white growths was allowed to pass through the granular carbon bed allowing a contact period of 36 minutes. The results of this experiment will be found in Tables IX, X and XI.

TABLE IX.

Cold Weather : 15-10-33 *to* 28-2-34. (H_2S *production in the control slow sand filter was at its minimum.*)

	Raw Water	Slow Sand Filter at 4" v.p.h.	Carbon Contact Bed at 7 gallons per Hour
Total count per c.c. ..	1300	190	300
Lactose fermenters (grade) ..	5·4	2·9	2·5
Colour (do.) ..	3·0	2·8	1·0
Clarity (do.) ..	2·8	1·4	1·0
Taste and smell (do.) ..	2·0	3·6	1·0
Free ammonia (parts per 100,000)	0·009	0·010	0·002
Albuminoid ammonia (do.) ..	0·027	0·018	0·006
Oxygen absorbed (do.) ..	0·141	0·139	0·037

TABLE X.

Hot Weather : 1-3-34 *to* 30-9-34.   (*When* H₂S *production was at its maximum in the slow sand filter.*)

		Raw Water	Slow Sand Filter at 4″ v.p.h.	Carbon Contact Bed at 7 gallons per Hour
Total count per c.c.	.	1040	295	109
Lactose fermenters (grade)	.	5·4	2·7	2·0
Colour	(do.) .	3·2	2·7	1·0
Clarity	(do.) .	1·5	1·1	1·0
Taste and smell	(do.) .	3·2	4·0	1·0
Free ammonia	(parts per 100,000)	0·002	0·013	0·002
Albuminoid ammonia	(do.) .	0·034	0·018	0·010
Oxygen absorbed	(do.) .	0·175	0·226	0·047

TABLE XI.

Cold Weather : 1-10-34 *to* 1-2-35.   (H₂S *production was at its minimum.*)

		Raw Water	Slow Sand Filter at 4″ v.p.h.	Carbon Contact Bed at 7 gallons per Hour
Total count per c.c.	..	988	140	106
Lactose fermenters (grade)	..	6·0	3·8	1·5
Colour	(do.) ..	3·0	2·6	1·0
Clarity	(do.) ..	2·6	1·5	1·0
Taste and smell	(do.) ..	2·0	3·7	1·0
Free ammonia	(parts per 100,000)	0·005	0·012	0·001
Albuminoid ammonia	(do.) ..	0·033	0·015	0·008
Oxygen absorbed	(do.) ..	0·149	0·210	0·044

These results clearly prove the remarkable efficacy of granular, activated carbon in rendering a repulsive looking and evil smelling water, æsthetically perfect (sparkling, clear, colourless, tasteless and odourless) and, in addition, effecting a general all-round improvement in quality (75 per cent. reduction of organic matter) such as cannot be attained with the methods of purification so far tried at Kilpauk.

General Discussion.

The great adsorptive powers of activated carbon and its consequent usefulness in water purification have been established beyond doubt. The choice of the form of carbon to be used will largely depend on the point of application and type of plant and possibly also to some extent, on the quality of the raw water.

The cost of treatment with powdered active carbon in America has been variously estimated at between 1 and 2 dollars on an average per million gallons treated. Accurate figures for granular carbon are not available and will probably not be available for a few years more, as the cost will depend largely on the effective life of the granular carbon beds. We have ventured to work out the relative costs on the basis of wholesale prices per ton quoted for the brand of carbon we used in these experiments.

Powdered active carbon applied to the water derived from the City supply mains, at 3 to 3·5 p.p.m. for the removal of colour, taste and odour, would cost between Re. 1 and Rs. 1-8-0 per million gallons.

Granular active carbon, if used alone in contact beds to a depth of 18 inches for the removal of tastes and odours in the effluent from a properly worked slow sand filter allowing a contact of about 5 to 6 minutes (2 to 3 minutes is considered sufficient by many) would cost about Rs. 8 per million gallons (on the basis of the wholesale price of Rs. 1,450 per ton of normal granular carbon with an effective life of *not less than* three years).* The annual expenditure then, in treating the Madras City supply, would be about rupees sixty thousand or slightly more, depending on the effective contact period. If, after three years, the carbon still retains potency or can be revivified into activity for a further period of one or two years at reasonably cheap rates, the annual cost of the treatment would be proportionately reduced.

From the data available with us, it would appear that the activated granular carbon used in our experiments, since September 1933, has not

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* We have since tested a different brand of granular activated carbon which appears to be at least as efficacious and is considerably cheaper.

so far suffered any appreciable deterioration in quality—as evidenced by the continued good results obtained from the analyses of the effluents from the carbon units. Beyond the ordinary cleaning and relaying of the carbon layers whenever the sand filters clogged, no special treatment was given to the carbon in the direction of revivification.

In the event of this method of treatment being found the most suitable one for any water, it would be very economical to instal a plant for manufacturing activated carbon as there is no dearth of the raw materials in India. The locally made product would thus be very much cheaper than the imported one. Used carbon could, in this case, be taken to the factory for revivification and brought back into use at the filters, at a nominal cost.

In this method then, there is the possibility of yet another solution to the Madras water supply problem. The successful use on a small scale of granular carbon for the removal of tastes and odours and for producing, at the same time, an æsthetically excellent supply (from an initially unsatisfactory filter effluent charged with H_2S) naturally leads us to commend the application of the process to all water supplies with taste and odour troubles such as are found in the Madras City supply.

From the experience gained in the application of activated carbon to the Madras City supply, it is suggested that a small and inexpensive plant containing activated carbon could be installed in individual homes, to obtain an æsthetically good product from the water as supplied through the taps. An earthenware cylinder 18″ high and 3″ to 6″ in diameter, holding a layer of 12″ of activated granular carbon, and provided with an outlet and stop-cock, would constitute the whole plant. Cylinders of copper or other metal are liable to corrosion by the activated carbon and are therefore not suitable containers for this purpose. The tap water may be allowed to drip slowly on to the surface of the carbon in the cylinder and percolate through the layer to the outlet, whence it is collected for use.

Summary.

Experiments on a small scale, using powdered and granular activated carbon for the removal of tastes and odours present in the Madras City water supply, were carried out between February 1933 and February 1935. The experiments were designed (1) to remove the colour, taste and smell found in the treated water as supplied to the City, (2) to prevent the formation of these undesirable features by filtering the raw water through a slow sand filter having a thin layer of carbon in its composition, and (3) to test the potency of the carbon in dealing with a slow sand filtered water heavily laden with H_2S and filamentous growths and having pronounced tastes and odours.

Conclusions.

1. Activated carbon (both powdered and granular) is effective in removing the colour, taste and odour from the treated water as supplied to the City. The treatment with the granular carbon resulted in the organic matter being reduced to a greater extent than with powdered carbon.

2. When used in a slow sand filter (as a sandwiched layer $1\frac{1}{2}''$) for filtering the lake water, granular activated carbon had a distinct sphere of usefulness. It was found that the filter containing the carbon layer yielded a better effluent than the control filter.

3. When used as a contact medium for the removal of colour, tastes and odours formed in a carefully controlled experimental slow sand filter effluent, activated granular carbon gave very satisfactory results.

4. The granular carbon used in our experiments since September 1933 has not shown any evidence of deterioration in quality after 23 months of continuous service.

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THE CONGLOMERATES AND GRITS OF KALDURGA, KADUR DISTRICT, MYSORE.

BY CHARLES S. PICHAMUTHU, B.Sc., F.G.S., F.R.S.E.,

(From the Department of Geology, University of Mysore, Bangalore.)

1. *Introduction.*

SIXTEEN years ago, the writer had the opportunity of examining parts of the Kaldurga area, especially the conglomerates occurring therein, in the company of the late Professor P. Sampat Iyengar, than whom one could not expect a better exponent of the autoclastic origin of the conglomerates of Mysore. He was struck then by the size and degree of rounding which many of the pebbles exhibited and wondered whether pressure alone could have been the cause for the formation of such a sedimentary looking conglomerate. In 1932, he visited parts of the Kadur District, when a halt was made at Jodikatte, and the conglomerates and associated rocks were examined in detail, and a large collection of the conglomerate and the included pebbles was made. The material was studied in the Geological laboratory of the University of Glasgow, and this paper is the record of work which was mainly done there. The writer visited the area once again this year and was able to observe several new features presented by the conglomerates and grits, which are incorporated in this paper.

2. *Previous Work.*

Bruce Foote, while he was a Deputy Superintendent of the Geological Survey of India, made a traverse across the Mysore State, and has given the earliest description of these conglomerates. He noticed the rugged nature of the hills formed by these rocks, which, according to him, resemble "typical granitoid-gneiss hills". He describes the rock as having a coarse mottled structure due to the presence of "enormous numbers of well-rounded pebbles of a granite or compact granite-gneiss. The size of the included stones ranges from small pebbles to large boulders, all enclosed in a greenish-grey foliated chloritic matrix" (R. Bruce Foote, 1882, p. 195). He was later appointed as State Geologist in Mysore, and after his retirement, his observations were published by the Mysore Geological Department as a Memoir. In this work, he has divided the conglomerates into two series, a lower, composed of coarse shingle lying in a "schisty or clayey matrix" and an upper formed

of coarse shingle cemented by a "very strong sandstone matrix" (R. Bruce Foote, 1900, pp. 29-31).

A brief reference to these conglomerates was made by Wetherell while dealing with the Geology of the Tarikere valley. He noticed the resemblance which these bore to the conglomerates found further north in the Ajjampur area (E. W. Wetherell, 1903, p. 97).

Though Slater accompanied Bruce Foote to Kaldurga in the year 1896, it was only in 1906 that he gave any description of these conglomerates. As the result of his examination he found that pebbly beds alternated with very fine-grained bands. The pebbles were of granite, granophyre, keratophyre, hornblende schist, quartzite and ferruginous quartzite. Regarding the matrix he states, "the further the matrix of these conglomerates is studied microscopically, the more do they appear to be a phase of an igneous rock—the quartz porphyry" (H. K. Slater, 1906, p. 4). He considered that the north-eastern arm of the Kaldurga conglomerates if continued in that direction would connect the conglomerates on the northern side of Honnegudda ($\triangle$ 3338) of the Ajjampur area and that the two might be parts of the same formation, since the pebbles and the matrix were similar. The Kaldurga conglomerates had also a marked resemblance to those occurring near Jhandimatti between Bhadravati and Channagiri (Fig. 1).

Regarding the origin of these conglomerates, Slater was not quite decided as can be seen from the following quotation: "Where granite or quartz porphyry intruded other members, *i.e.*, intermediate lava flows of which numerous pebbles exist—pebbles of schist would result through pressure and movement, portions of both the intruding and intruded rock being finely comminuted, would go to form either a felspathic and gritty or chloritic and calcareous matrix. The variety of fragments—granite, quartz-porphyry, quartzite and keratophyre—may be accounted as fragments of the adjacent schist which have been thus fractured and penetrated by the granite or quartz-porphyry, and the whole subsequently crushed. But they might equally well represent a sedimentary grit" (H. K. Slater, 1906, p. 5).

It was, however, ten years later, that a decisive opinion regarding the origin of these conglomerates, was given. Dr. Smeeth, who was the Director of the Mysore Geological Department, after an examination of the Kaldurga area arrived at the conclusion that the conglomerates were autoclastic and not sedimentary, though he admits that "a few portions which show very rounded pebbles of quartzite in a schistose matrix are difficult to explain" (W. F. Smeeth, 1915, p. 25). He considered it probable that the Ajjampur conglomerates corresponded with those of Kaldurga, the two exposures being separated by the gneiss and granite of the Tarikere valley.
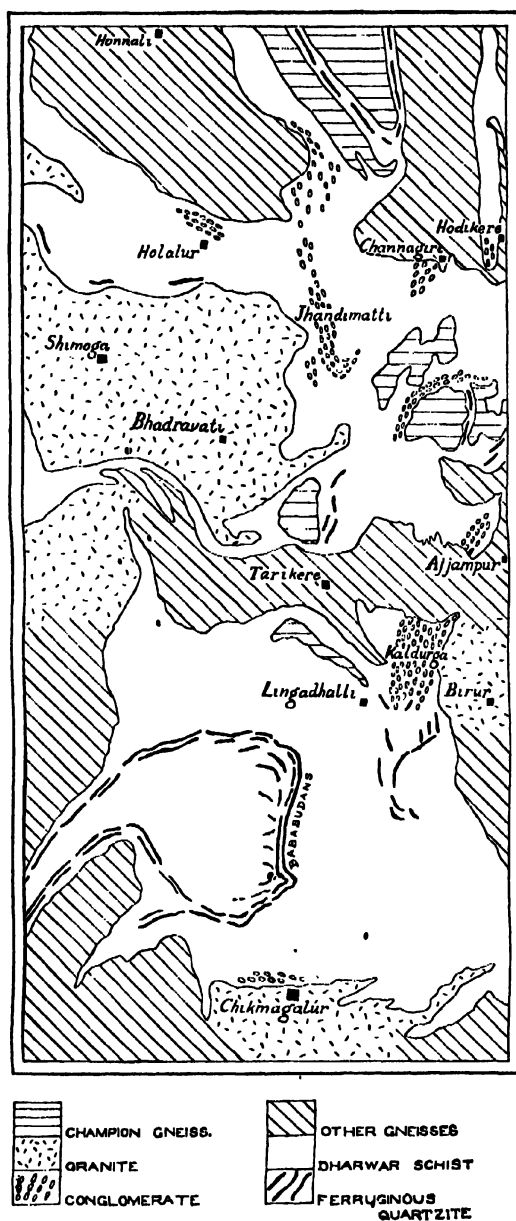
FIG 1. Geological sketch map of a portion of the Shimoga schist belt, showing the distribution
of the conglomerates.

In 1916, Sampat Iyengar made a brief reference to these conglomerates, while describing the schistose rocks of the Bababudan area (P. Sampat Iyengar, 1916, p. 134), but it was in the following year that he undertook a detailed study of these rocks. He emphasised the autoclastic origin of the conglomerates which he considered as "a mixture of various complexes brought about by crush and shear movements operating on the previously existing schist rock and on the igneous intrusives into them" (P. Sampat Iyengar, 1917, p. 115). He was of opinion that the brownish gritty mica chlorite schists and the dark green chlorite schists were the crushed and altered phases of the Champion gneiss which is intrusive into pre-existing hornblende schists, amphibolite and some chlorite schists. Into this complex the Tarikere granitic gneiss is supposed to have intruded, tongues of this gneiss which penetrate the schists having been converted along shear zones into conglomeratic masses. He collected pebbles of granite, reef quartz, quartzite, dark green chlorite schist, limestone, amphibolite and hornblende schist.

In all previous reports dealing with this and, with the adjacent Ajjampur area, the opinion was expressed that the Kaldurga and Ajjampur conglomerates formed one continuous formation which was later interrupted by the intrusive gneissic granite of Tarikere. Sampat Iyengar did not agree with this view since he considered the Kaldurga conglomerates and the Tarikere gneissic granite as one and the same. According to him, "it is quite probable that the Tarikere gneissic granite at its northern end also did send into the schists several tongues which like their compeers in the south have also become autoclastic conglomerates through the operation of the same forces of nature" (P. Sampat Iyengar, 1917, p. 116).

In his annual report of the work of the Mysore Geological Department for the year 1916, Smeeth endorsed the opinions expressed by Sampat Iyengar, regarding the origin of the conglomerates. He agreed that "the bands of intrusive gneiss, the crushing and shearing of which have given rise to much of the conglomerate, are not distinguishable from the gneiss of the Tarikere valley" (W. F. Smeeth, 1917, p. 33). This view of Smeeth and Sampat Iyengar regarding the identity of the conglomerate and the Tarikere gneiss has, however, been contradicted by Jayaram, who, during the revision survey work, examined the junction of the Tarikere granite with the conglomerate series of Ajjampur and found that "the granite proper which tongues into the schistose series does not form part of the conglomerate series" (B. Jayaram, 1923, p. 26).

During the field season of 1918-19, Jayaram examined these conglomerates. He describes them as "granite-porphyry conglomerate", "intermediate granite-porphyry conglomerate", and "chlorite-keratophyre-schist

conglomerate"; these types are supposed to be gradational, one merging into the other. The "granite-porphyry conglomerate" is, according to him, "very instructive for studying the pebble formation in an igneous rock, which must have had a viscous flow condition favourable for the formation of phenocrysts, knots, acid and basic segregations and schlieren that have on subsequent shearing assumed the conglomerate form" (B. Jayaram, 1922 b, p. 64). The term "grit" has been used in this report several times but without any sedimentary significance as he considers it "a variant of the micro-granite".

In his Presidential address to the Geology Section of the Indian Science Congress at Nagpur, Sampat Iyengar stated that at its contact, the Peninsular gneiss has imposed an autoclastic structure on the Champion gneiss (P. Sampat Iyengar, 1920, p. 7).

No further work has been done since then on these conglomerates, and so the autoclastic theory remains at present the official opinion of the Mysore Geological Department regarding the origin of the Kaldurga conglomerates. Middlemiss has remarked on the unanimity with which this view has been advocated by the Mysore geologists in his Presidential address to the Geology Section of the Fourth Indian Science Congress held in Bangalore (C. S. Middlemiss, 1917, p. cxcvi).

3. Geology of the Area.

Before describing the conglomerates and grits, it is proposed to give a brief account of the geology of the area. The Kaldurga conglomerates form part of the Shimoga belt of the Dharwar schists. The schist belt in the neighbourhood of the conglomerates is composed of epidiorite, spilite, keratophyre, chlorite schist, calc-chlorite schist, mica-chlorite schist, grits, quartzite and magnetite quartzite. Small outcrops of limestone, amphibolite and talc schist occur. Intrusive into' the schists are quartz veins, Champion gneiss, Peninsular gneiss, Kaldur granite and dolerite dykes.

Epidiorite.- A portion of the Lingadhalli traps appears at the extreme south-west of the area under consideration. This epidiorite varies in texture from a very fine-grained and tough rock to a coarse one exhibiting poikiloblastic structures. The outcrops of this rock about half-a-mile north-east of Lingadhalli abound in rounded or oval "spots" which were considered by Slater to be "pseudo-amygdules due to the crushing of quartz phenocrysts" (1906, p. 12) or *in situ* alterations of "porphyritic crystals of hornblende and not true amygdules (H. K. Slater, 1908, p. 48). According to Sampat Iyengar, some of them were *in situ* alterations of plagioclase phenocrysts and some due to the brecciation of quartz or epidote veins

(P. Sampat Iyengar, 1908, p. 70). A detailed study of these rocks by the writer has shown that these "spots" are true vesicles filled in by various secondary minerals (C. S. Pichamuthu, 1932, pp. 127-137). An interesting feature of some of these amygdales is the occurrence of plagioclase felspars as infilling minerals. It is probable that these have been formed by the alteration of some zeolitic mineral which might originally have occupied these cavities (C. S. Pichamuthu, 1933, p. 345).

The epidiorite is composed essentially of a pale green hornblende which is often fibrous. Plagioclase, when present, is of an acid variety. A small quantity of quartz is usually present. Granular and idioblastic epidote is common, as is also ilmenite, invariably altered to leucoxene. Some varieties contain abundant chlorite.

Spilite, Keratophyre.—As the result of the examination of the rocks of this area, both in the field and under the microscope, the writer is of the opinion that there is a spilitic suite of rock characterised by the presence of albite and acid oligoclase. Chemical analyses of some of these types are under progress and when they are complete, it is hoped that the relationships of the different rocks occurring here, will be better known. Some of the rocks which have been mapped as the Lingadhalli traps are spilitic. About half-a-mile south-east of $\triangle 3179$, a vesicular spilite occurs. The microsections of this rock are stippled throughout with fine leucoxenic grains, which are specially concentrated around the amygdales. Felted aggregates of fibrous hornblende and laths of albite form the main constituents. The amygdales are filled by a pale green pleochroic epidote, quartz and chlorite. The epidotes exhibit a peculiar form of corrosion. Sometimes circular portions of the epidote are removed and replaced by quartz ; in other cases, a remnant of the epidote is left in the centre of these circular areas. The edges of the epidote which have thus been attacked exhibit a scalloped outline.

The term " Keratophyre" has been used in a very loose sense in many of the reports of the Mysore Geological Department, to describe rocks which have no textural or mineralogical resemblance to the type variety. It has practically come to be synonymous with a calc-chlorite schist. In this paper, the term is confined to those rocks which contain laths of albite and which exhibit a trachytic or bostonitic texture ; in some cases where the albite laths are not flow-oriented, the texture approaches that of a basalt or fine-grained dolerite. Chlorite occurs in varying proportions. Calcite is invariably present. Very good exposures of this rock were noticed by the writer on the flanks of the small conical hill, about half-a-mile south-east of Lingadhalli. The rock is dark in appearance but the specific gravity is as low as

2·68. Overlying the keratophyres here, exceedingly fine examples of albite schists occur. Porphyroblasts of clear albite are found in a sericite chlorite matrix.

Amphibolite, Talc-schist.—Exposures of amphibolite and talc-schists are seen near Jodikatte. The amphibolites are composed mainly of a pale hornblende. Grains of calcite and iron ore are common. The talc-schists occurring outside the conglomerate area and adjoining the granites, probably represent shear zones.

Limestone.—A small patch of impure limestone outcrops to the south-west of Jodikatte. It contains fuchsite and is traversed by numerous thin veins of quartz. The rock is very rich in inclusions of rutile.

Quartzites, Banded ferruginous quartzites.—There are numerous runs of quartzites associated especially with the chlorite schists. They conform generally to the directions of strike and dip of the schists. They are composed essentially of a granoblastic aggregate of quartz grains. Chlorite occurs frequently; fuchsite was noticed in a few runs of the quartzites near Jodikatte. Tourmaline is sometimes present. Muscovite, biotite, pyrites and magnetite are other minerals which occur in the quartzites. Some of the quartzites show faint traces of banding and this becomes conspicuous as the iron content increases. Typical banded ferruginous quartzites occur on the hills to the east of Lingadhalli and on the small rise near Huvinhalli.

Gneiss.—The oldest gneiss occurring in Mysore has been called the Champion gneiss. A strip of this is seen to the north of Lingadhalli. In the map of Mysore published by the Geological Department, the conglomerates are also shown as Champion gneiss, a view with which the writer finds it difficult to agree. The Champion gneiss is considered to be intrusive into the Dharwar schists but the term "Champion gneiss" has been used in Mysore with such different meanings and to include so many types of rocks that it is difficult to comprehend what exactly is meant by it. A good idea of the various rock types which are supposed to be allied to the Champion gneiss, may be obtained from Sampat Iyengar's Presidential address to the Geology Section of the Indian Science Congress (P. Sampat Iyengar, 1920, pp. 2-13). The Peninsular gneiss is later in age than the Champion gneiss and includes the gneissic granites of the Tarikere valley, as well as the gneisses to the east of the conglomerate area.

Granite.—The Kadur granite is intrusive into the Peninsular gneiss. The intrusion of this granite has been accompanied by pneumatolytic action resulting in tourmalinisation and greisening. Examples of schorl and greisen were noticed by the writer about half-a-mile north-west of Jodikatte.

Granodiorite.—An outcrop of this rock occurs on the flanks of the hill north of the 4th milestone on the Birur to Lingadhalli road. Its relationship with the other rocks was not clear. Big grains of quartz are present, showing undulose extinction. The plagioclase which is mainly albite, occurs as large crystals ; the crystals are invariably bent or broken, the fractures being healed by chlorite and iron ore. There is abundant biotite undergoing alteration to chlorite. Apatite is the chief accessory.
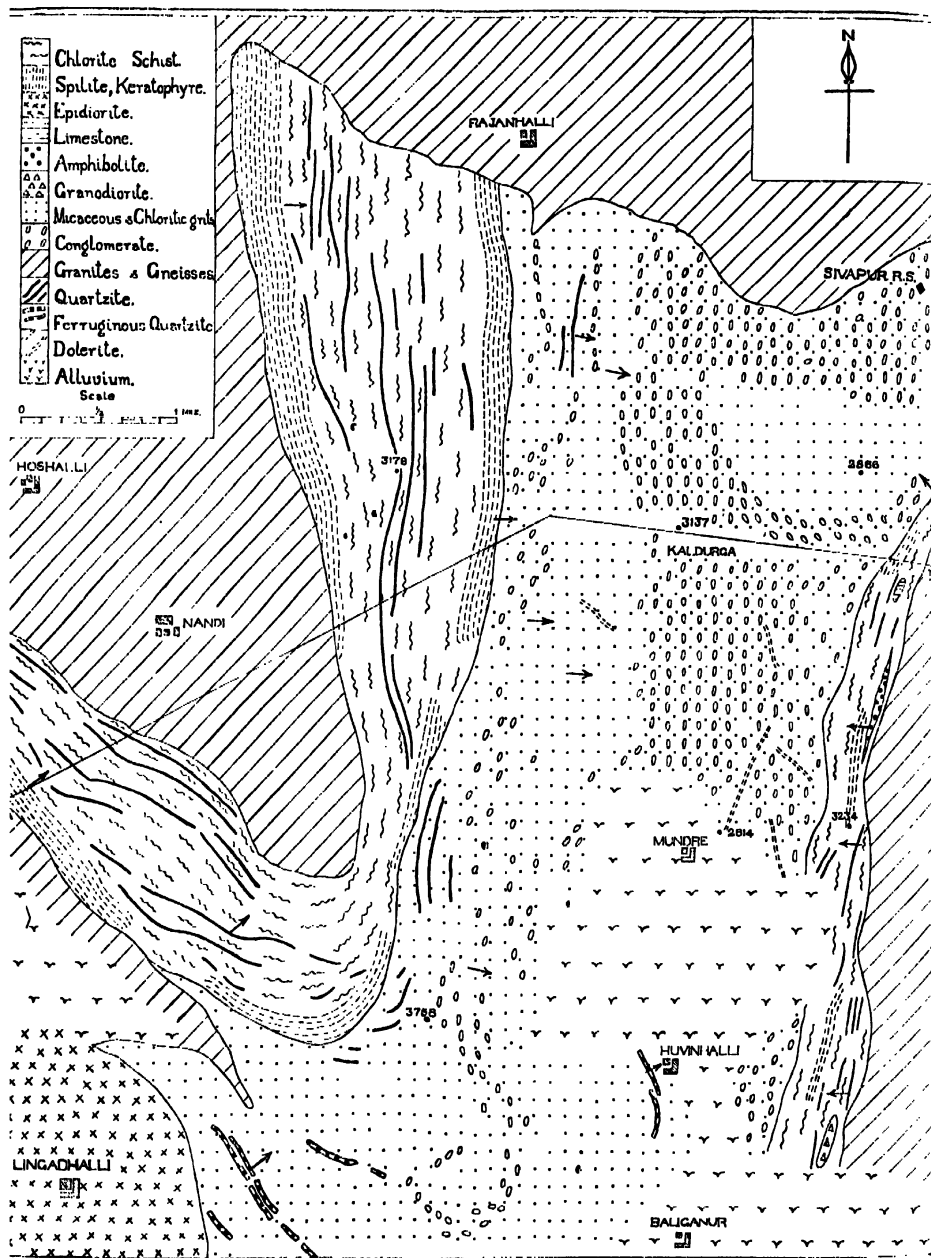
Vein quartz.—There are many runs of massive white quartz. These are related to the different acid intrusives and are not therefore all of the same age.

Dolerite.—A few dykes of normal dolerite occur in the area.

4. Tectonics.

The geological structure of the area as well as the general succession of the rocks occurring here, may be seen from the map (Fig. 2) and the section (Fig. 3). The oldest rocks are the chlorite schists and mica chlorite schists. Spilites, keratophyres, quartzites and banded ferruginous quartzites occur interbedded with these schists. The schists pass upwards into grits and conglomerates. The occurrence in the conglomerates of pebbles of granite and gneiss, which differ from the granites and gneisses of the area in the absence of potash felspars, raises an interesting problem. These pebbles must have been derived from rocks which are older than the conglomerates but so far the writer has been unable to trace any outcrops of these rocks. The abundance of acidic rocks in the upper chloritic division is difficult of explanation if they are considered to be derivatives of the Champion gneiss and hence later than both divisions of the Dharwars. Smeeth therefore suggested that some of them should be "associated with a period of granitic intrusion still earlier than the Champion gneiss, but of which period the primary granite or gneiss has not been identified and separated. Remnants of the earlier gneiss might easily remain amongst the very varied types which are at present included under the designation 'Champion gneiss' " (W. F. Smeeth, 1921, p. 158). It is probable that these pebbles discovered by the writer in the Kaldurga conglomerates, are derived from this 'Pre-Champion gneiss'. The Peninsular gneiss is intrusive into the schists and the Kadur granite intrudes this gneiss. Dolerite dykes are the youngest rocks in the area.

The dip of the rocks is uniformly towards the east, except near the eastern margin of the area, where the beds dip westward. There is an overfold in the western portion, by the denudation of which the Nandi-Hoshalli valley has been formed. The beds are folded into a syncline on the east.

Legend:
- Chlorite Schist.
- Spilite, Keratophyre.
- Epidiorite.
- Limestone.
- Amphibolite.
- Granodiorite.
- Micaceous & Chloritic gnb
- Conglomerate.
- Granites & Gneisses.
- Quartzite.
- Ferruginous Quartzite.
- Dolerite.
- Alluvium.

Scale
0 _____ 1 mile

(Modified by the writer from the map of Sampat.Iyengar, *Recs. Mys. Geol. Dept.*, Vol. 15.)

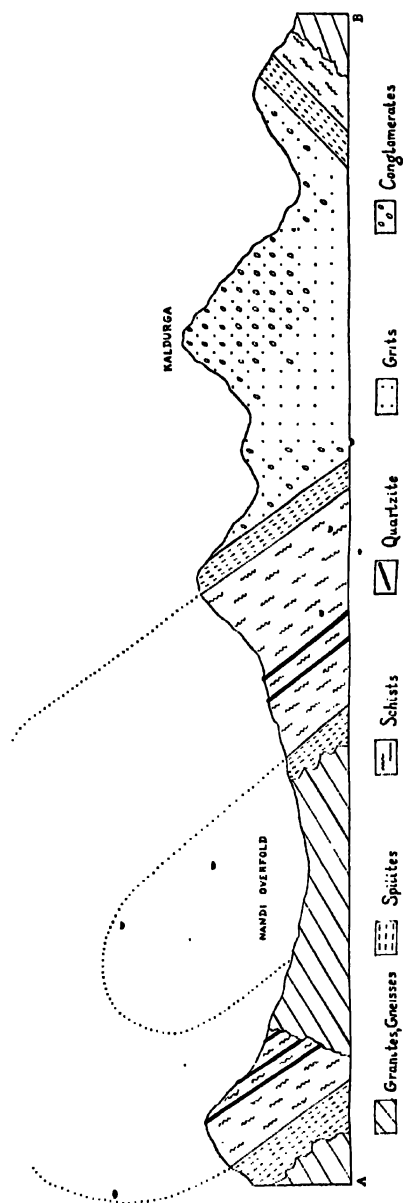FIG. 2. Geological sketch map of Kaldurga and surroundings.

FIG. 3. Section along AB in Fig. 2.

5. *The Conglomerates and Grits.*

The conglomerates and grits overlie the chloritic schists and the associated spilites and keratophyres. Sampat Iyengar, in his map of this area, has differentiated chlorite schists and brownish mica chlorite schists. Though in places one comes across brownish coloured schists and in other places dark green chlorite schists, the boundary between the two is not so sharp as has been represented on his map. Under the microscope, many of his "brownish mica chlorite schists" show very little mica ; they are composed almost entirely of chlorite.

The conglomerates and grits can, however, be divided into two series : a lower, characterised by the abundance of chlorite, and an upper, which is more gritty and in some cases felspathic. The lower division may be described as a greywacke ; the upper varies from quartz schist to a felspathic grit or arkose. The lower division is typically schistose, the strike and dip being ordinarily well marked. In contrast to this, the exposures of rocks belonging to the upper division are bouldery, and have the characteristic appearance of granitic outcrops. Many of the huge boulders stand out as prominent tors and form the range of hills from Rajanhalli to Mundre.

The lower chloritic schists and grits present no unusual features. Biotite has frequently been formed from the chlorites. Due to crushing these schists often possess, under the microscope, the typical appearance of a sheared grit (Plate XXIV, Fig. 1), the quartz grains forming tiny "eyes" and the flaky minerals encircling them. Amber yellow rutile grains are often abundant.

In the conglomerates of the upper division, it is not uncommon to see pebbly bands alternating with very fine-grained non-pebbly bands. To a casual observer these bands suggest rocks of different composition, but an examination of sections cut from them proves their identity. The finer bands represent the material which serves as the matrix in the pebbly layers. In some cases chlorite, biotite and muscovite are set in a granoblastic matrix of quartz. The flaky minerals vary in proportion ; when they are abundant, a fairly regular parallel arrangement is seen and the rock may then be called a quartz schist. Very often, grains of plagioclase felspar, usually albite or acid oligoclase, are present ; but microcline was not observed at all, and since this is the most resistant of the felspars to alteration, it is to be concluded that the mineral never occurred in these rocks.

Extremely fine varieties of grits are seen on the hills about a mile west of Baliganur. The outcrops in this place exhibit graded bedding. The rock here has layers about a foot in thickness one overlying the other. Each

layer starts with a more or less coarse pebbly character and gradually becomes finer and finer, till the next layer again starts abruptly with coarse pebbles, grading on into fine (C. S. Pichamuthu, 1935, p. 431). In some cases a crude current bedding was observed which also exhibited graded bedding.

The coarse grits collected here proved very interesting material for study. In hand specimens, they are found to be composed of small fragments of various types of rocks. This character is very well seen under the microscope (Plate XXIV, Fig. 2). Many of the individual grains in the grit are composed of quartzite, but small fragments of granite, spilite, keratophyre, phyllite and vein quartz are also seen practically in every section. The rock has been subjected to pressure, resulting in many of the fragments being flattened and drawn out, but their boundaries are quite distinct and do not merge with the matrix.

6. The Pebbles.

The conglomerates contain pebbles of various sizes. In some cases they are as much as 12″ to 18″ in diameter, and they grade down into very small sizes in the pebbly grits. In certain areas, pebbles are abundant, and in others, their occurrence is sporadic. In the same outcrops it has been noticed that some bands are very rich in pebbles and that they alternate with bands which do not contain any. The pebbles are of various shapes but almost every one of them exhibits some degree of polishing. Oval and rounded pebbles are of frequent occurrence.

The conglomerate is often crowded with a large number of pebbles of a varied petrographical character. Plate XXIII is a photograph of a specimen about 6″ × 9″ in size, obtained from the south of Kaldurga. It contained nearly thirty pebbles composed of such different types as gneiss, granite, microgranite, pegmatite, quartzites (two varieties), quartz schist, quartz-felspar porphyry, and vesicular spilite. The following description of the pebbles collected from the Kaldurga conglomerates illustrates the great variety of the rock types which have supplied the pebbles.

Granite.—Pebbles of granite have been reported to occur by practically every geologist who has visited these conglomerates. The writer has examined the slides of pebbles collected by himself, as well as those preserved in the museum of the Mysore Geological Department, and in all these cases, the felspar is albite or acid oligoclase. No orthoclase or microcline is met with. Quartz and biotite (which is sometimes altered to chlorite) are present. Such rocks have been designated soda-granites, but since this term has also been applied to granites in which there is more soda than potash, and since quartz bearing acid plagioclase rocks are tonalites, Johannsen prefers to call these plutonic rocks, which are granite-like in appearance but contain only

a trace or no potash felspar, as sodaclase-tonalites (A. Johannsen, 1932, p. 373).

Gneiss.—The light-coloured pebbles with gneissic banding, also do not contain orthoclase or microcline. The essential minerals are albite, quartz and biotite. Muscovite is commonly present. Apatite, zircon and rutile are the chief accessory minerals. Some of the sections exhibit a diablastic structure due to albite-quartz intergrowths. The biotite is frequently altered to chlorite; that the mica is titaniferous is evidenced by the sagenitic webs of rutile sometimes found inside the chlorite flakes. The green chloritic mineral present in some sections of gneiss, and which exhibits maximum absorption parallel to the vibration direction of the polariser, may be intermediate between mica and chlorite; it usually has a well-marked pleochroism from yellow to green. Some of the pebbles contain abundant epidotes while others contain none. The felspars are often bent or broken. In highly crushed varieties, the rock is mylonised and the quartzes are converted into a mosaic of small grains.

Pegmatite.—Albite and quartz occur intergrown. Very little biotite and chlorite are present. As the result of crushing the flaky minerals are sometimes found as streaks.

Aplite.—These are fine-grained and equigranular, exhibiting under the microscope a typical allotriomorphic texture. It is composed mainly of albite and quartz, chlorite being in very subordinate amount. The felspars are sericitised.

Microgranite.—The minerals present are albite, quartz and biotite. Grains of magnetite are scattered throughout the sections. The texture is microgranular.

Granodiorite.—Albite occurs in long crystals, which are invariably bent or broken; when broken, chlorite fills the interspaces between the fragments. No biotite was noticed, as it has probably all been converted into chlorite. The chloritic patches contain grains of leucoxene. The pebble is highly crushed and traces of a diablastic texture are present. It is comparable to the rock noticed by the writer on the slopes of the hill north of the 4th milestone between Birur and Lingadhalli, and of which a brief description has been given earlier. The only difference is in the occurrence of biotite in the rock and its absence in the pebble.

Granophyre.—The pebbles are composed chiefly of big plagioclase felspars which show a very characteristic intergrowth texture along the borders (Plate XXIV, Fig. 3). The rock may be described as a plagioclase granophyre or markfieldite.

Amphibolite.—This is a very coarse rock composed almost entirely of a pale hornblende. Grains of calcite and iron ore are present.

Hornblende schist.—This is a coarse diorite-like rock consisting of plagioclase and pale hornblende. This seems to resemble the recrystallised rock described as interaction diorite from near Karehalli. Biotite occurs in small quantities. Ilmenite altering to leucoxene is common. Chlorite and zoisite occur as secondary minerals.

Serpentine.—The pebble was obtained about a mile west of the Sivapur Railway Station. It is composed wholly of serpentine with abundant magnetite dust. The original nature of the rock is not clear. It is traversed by veins of calcite. The specific gravity of the pebble is 2·70.

Quartz felspar porphyry.—Albite occurs as phenocrysts in a very finegrained and compact matrix (Plate XXIV, Fig. 4). Orthoclase phenocrysts are rare. Areas of coarse quartz mosaics represent crushed phenocrysts of quartz.

Felsite.—This is a very fine-grained crystalline rock. Albite occurs as phenocrysts, many of which are broken due to pressure. These crystals are almost always bordered by a zone of chlorite which has well marked pleochroism from yellow to green. Quartz sometimes occurs in granular mosaics. The compact matrix contains abundant flakes of chlorite, and is peppered with numerous minute magnetite crystals. Acicular apatites occur as accessory minerals. This pebble strongly resembles the rock described as felsite from Galipuje (13° 27' : 75° 47') (P. Sampat Iyengar, 1908, p. 78), but unlike the typical rock, it contains no biotite, the chlorite and magnetite being derived from its alteration. The specific gravity of the pebble is 2·59.

Rhyolite.—The pebble is composed of a dark grey compact rock. Under the microscope it is seen to be highly crushed and sheared. The fine-grained matrix is formed of quartz and a pale chlorite. Fan-shaped aggregates of felspar occur, often in rows; the way in which they extinguish indicates that they are crushed and drawn out spherulites or sectors of spherulites. Minute grains of rutile are common as accessory. The specific gravity of the pebble is 2·69.

Keratophyre.—Some of the pebbles have a typical trachytic texture composed of numerous laths of albite. Biotite is the chief mafic mineral. A little chlorite and occasional rhombs of calcite are present. In other pebbles of this rock, more chlorite than biotite occurs, with scattered crystals of magnetite.

Albite dolerite.—These exhibit a hyalo-ophitic texture with albite laths as the principal mineral. Excepting for this type of texture, the rock seems to be allied to the keratophyres described above.

Spilite.—This is a vesicular rock with albite as the chief variety of felspar. Chlorite, biotite and muscovite (which may be paragonite), occur. There are numerous tiny grains of magnetite. A little quartz is present. There are only slight differences between this and the albite dolerites and keratophyres.

Quartzite.—The pebbles are formed of granoblastic aggregates of quartz (Plate XXIV, Fig. 5). A mosaic texture is more common, but in some varieties a sutured texture is noticed. Some of the bigger grains of quartz exhibit peripheral granulation. The cementing material has gone into chlorite and this mineral often outlines the grains of quartz. Biotite and muscovite sometimes occur in small quantities. Cubes of pyrites and grains of magnetite, tourmaline and rutile are occasionally present.

Quartz schist.—These are rocks which contain no felspar and are formed essentially of quartz. There is a banded texture, layers of quartz being separated by streaks composed of biotite, chlorite, muscovite and epidote. Rhombs of a carbonate occur, and since they are gone into brown iron ore, the original mineral may have been siderite.

Magnetite quartzite.—Several pebbles of banded quartzite have been collected by the writer from the conglomerate area, but they are not the typical highly ferruginous banded quartzites which outcrop strongly on the Bababudans or on Dodbetta, near Lingadhalli. They are essentially composed of quartz mosaics but with a distinct banding which is accentuated by the segregation of tiny crystals of magnetite (Plate XXV, Fig. 1). These seem to be a sort of intermediate stage between the pure quartzites and the banded ferruginous quartzites. Tiny flakes of chlorite and small grains of tourmaline have been noticed in some sections. The specific gravity of one of the pebbles was found to be 2·70.

Phyllite.—The writer has noticed pebbles of phyllite on the hills west of Baligamur, which clink like slates when struck by a hammer. They are highly fissile and are composed mainly of sericite and chlorite.

Limestone.—The pebbles are formed chiefly of calcite (Plate XXV, Fig. 2). The rock sometimes possesses a blastoporphyritic texture, the porphyroblasts of calcite often exhibiting a diablastic intergrowth with quartz. A green chlorite, pleochroic in shades of yellow and green is common. Quartz occurs in minute granular aggregates. Grains of ilmenite and magnetite are sometimes abundant. Clusters of tiny yellow rutile crystals are present; these are often intimately associated with the ilmenite grains. This combination is somewhat similar to what has been observed by Dr. Fermor in the mica schists of Balaghat, which according to him are metamorphosed sediments (L. L. Fermor, 1909, pp 313-14).

7. *The Matrix.*

The matrix of the Kaldurga conglomerates is not quite uniform in composition throughout the area. As has already been indicated, it grades from chlorite and mica-chlorite schists, through gritty chlorite and mica schists, to quartz schists. In the upper division, the matrix may in some places be designated as an arkose because of the presence of grains of albite. Some of the grits which contain a good deal of chloritic and mica-ceous minerals could be described as greywackes. Ferruginous material is abundant in some cases. Calcite occurs in grains and in irregular patches. Minute rutiles are fairly common, especially when the matrix contains plenty of chlorite and mica. Muscovite is very often present (Plate XXV, Fig. 3).

The boundary between the pebbles and the matrix is usually well marked. The pebbles when removed, leave smooth-walled depressions which are coated with a layer of chlorite. The pebbles and matrix can also be distinguished under the microscope by their mineralogical and textural differences (Plate XXV, Fig. 4) ; the boundary is often knife sharp and is sometimes indicated by a thin line of chlorite (Plate XXV, Fig. 5). It is only in the upper division where sometimes a gneissic pebble is in contact with matrix composed of a felspathic grit, that the differences are not quite appreciable. It is such sections that must have led several of the previous observers to consider the conglomerates as autoclastic, and to state that there was no difference between the pebbles and the matrix.

8. *Mode of Origin.*

The question of the probable mode of origin of the conglomerates of Mysore has had an interesting history. Till the year 1908, the conglomerates were considered to be sedimentary (R. Bruce Foote, 1882, p. 195 ; 1900, pp. 29-31 ; V. S. Sambasiva Iyer, 1899, pp. 89-90, 97 ; 1901, pp. 121-23. P. Sampat Iyengar, 1905, pp. 73-74 ; H. K. Slater, 1903, pp. 126-28 ; 1905, p. 20 ; 1906, pp. 3-7 ; W. F. Smeeth, 1899, pp. 162, 165 ; 1902, p. 18 ; 1904, pp. 20-21 ; E. W. Wetherell, 1903, p. 92 ; 1904, p. 24). Later, Dr. W. F. Smeeth, the Director of Geology in Mysore, examined the Mallapanhalli (14° 4' : 76° 40') and Aimangala conglomerates of the Chitaldrug schist belt during the field season of 1909-10, and pointed out certain evidences which, according to him, were in favour of their being considered as autoclastic in character and not of the nature of true sedimentary conglomerates (W. F. Smeeth, 1910, pp. 15-18, 34-35). This idea was soon extended to all the other conglomerates of Mysore ; everyone of these was shown to fit in with the 'autoclastic' theory and the original suggestion of some of these being sedimentary came to be completely abandoned (B. Balaji Rao, 1913, pp. 139-40 ; 1928, pp. 88-89 ;

B. Jayaram, 1910, pp. 180-81 ; 1916, pp. 93-94 ; 1922*a*, pp. 84-86 ; 1922*b*, pp. 54, 57-58, 64 ; B. Rama Rao, 1924, pp. 179-81 ; P. Sampat Iyengar, 1908, p. 72 ; 1912, pp. 54-56 ; 1916, p. 134 ; 1917, pp. 106-16 ; A. M. Sen, 1916, pp. 150-53 ; H. K. Slater, 1912, pp. 26-29 ; W. F. Smeeth, 1910, pp. 12-18, 25, 34 ; 1912, p. 38). So thoroughly did the officers of the Mysore Geological Department support the views of their chief, that Smeeth says in one of his annual reports, "I appear to have raised quite a hornet's nest of the latter type (autoclastic conglomerates) and I long for some one to find a simple satisfactory sedimentary conglomerate with nicely rolled, water-worn pebbles" (W. F. Smeeth, 1912, p. 38).

It would not be out of place to consider briefly the reasons which led Dr. Smeeth to attribute to the conglomerates of Mallapanhalli and Aimangala, an autoclastic origin, especially because of the very great influence his views have undoubtedly had on all later works in the Mysore State. Referring to Mallapanhalli, he says, "I was struck by the possibility that the matrix might prove to be of similar material to the pebbles or boulders, though more crushed, particularly in the case of the grey trap pebbles." In some places, he thought that there was a clear transition from uncrushed boulder to crushed matrix in one and the same material and in such cases he was of the opinion that "there can be no doubt that the whole mass was originally grey trap which, after developing spheroidal structure, has been somewhat crushed and sheared leaving uncrushed spheroids in a crushed schistose matrix". This is quite a valid argument and is one of the main reasons for considering any conglomerate as autoclastic in origin. But in Mallapanhalli, pebbles of vein quartz and limestone were also found, and no resemblance between these and the matrix could possibly be discovered. Smeeth got over this by regarding the quartz and limestone pebbles as "xonoliths or rounded eyes of secondary minerals".

This difficulty was more pronounced in the case of the Aimangala conglomerates, since these rocks are largely composed of pebbles of such varied character as granite, quartzite, hornblendic rocks and banded ferruginous quartzite, set in a dark grey and somewhat gritty matrix with some chlorite and mica. In referring to this conglomerate, Smeeth says, "it certainly has the appearance of a genuine sedimentary conglomerate from the varied and rounded character of the pebbles and the contrast which many of them afford to the matrix, and for a time, I allowed it to pass as such, although it was difficult to account for its position in the midst of a wide expanse of chlorite schists. Since then, a careful examination of the specimens...... has led me to entirely alter my view. I find that a large number of the pebbles are indistinguishable under the microscope from typical portions of

the matrix, both consisting of rounded to subangular grains of quartz in a matrix of clayey material more or less impregnated with brown oxide of iron ....I now regard the whole occurrence as being of granite origin and intrusive with the chlorite schists, the granite pebbles being lumps of harder material isolated and rounded by shearing and crushing, and the lumps of hornblendic rock and ferruginous quartzite being xenoliths in intrusive granite" (W. F. Smeeth, 1910, pp. 34, 35).

Similar arguments were advanced to explain the nature of the conglomerates elsewhere. In the Hodigere conglomerates occurring in the Shimoga schist belt, the pebbles were considered by Slater to be "the result of pressure on intrusive bands or sills of quartzite and granite", though on the same page he says that "the granite pebbles are of the Honnali granite variety and shew practically no crushing in the microsections" (H. K. Slater, 1912, p. 28). According to Jayaram, the pebbles in the Gangut conglomerates are "sheared and drawn out phenocrysts and autoliths in the rock" (B. Jayaram, 1922b, p. 55).

As has been mentioned earlier, it was Sampat Iyengar who examined the Kaldurga conglomerates in any detail. He was definitely of the opinion that the conglomerates were autoclastic. According to him, mineralogically there was not much difference to be noticed between the gritty mica chlorite schists and the granite matrix of the conglomerate. The brownish gritty mica chlorite schist and the dark green chlorite schist were considered by him to be the crushed and altered phases of the Champion gneiss, into which tongues of the Tarikere gneissic granite have intruded and got converted along shear zones into conglomeratic masses.

After a fairly intensive study of these conglomerates and grits, both in the field and in the laboratory, the writer has come to the conclusion that the autoclastic theory cannot be applied to explain the origin of these rocks. It is proposed to bring together in the following paragraphs, certain data, some of which have already been briefly touched upon in the preceding pages ; these facts cannot adequately be explained by the autoclastic theory but, on the other hand, they suggest a sedimentary origin to these rocks.

Shape and rounding of pebbles.—Almost every observer who has visited the Kaldurga area has remarked on the shapes of the pebbles and the degree of rounding which they exhibit (Plate XXIII). In fact, these were practically the main reasons which led the earlier geologists to consider these conglomerates as sedimentary.

Sharp boundary between pebbles and matrix.—From slightly weathered specimens the pebbles can be easily dislodged, when they leave smooth

depressions. The boundaries of the pebbles are usually clean and marked by films of chlorite. This is very well seen even under the microscope (Plate XXV, Figs. 4 and 5).

Varied assemblage of pebbles.—From the description which has already been given in this paper, it will be seen that the conglomerates contain a very varied assemblage of pebbles. In small hand specimens, there are sometimes as many as six different petrographic types occurring as pebbles. This has been explained by the supporters of the autoclastic theory as due to the crushing of a complex containing different rocks as xenoliths. While prepared to admit that some of these rock types can occur as xenoliths in granite, the writer fails to see how so many different kinds of pebbles could be found aggregated within the space of a few square inches. This implies the breaking up of the xenoliths and a bodily migration of the pebbles caused by thorough churning of the rock during or after the formation of the pebbles, assumptions which are extremely improbable.

Difference between pebbles and matrix.—There are well-marked differences between the pebbles and matrix. This is naturally to be expected when there are so many different types of rock represented among the pebbles. This difference is not easily noticed when pebbles of granite or gneiss are found in the upper division or when pebbles of schist occur in the lower division. But identity between pebbles and matrix cannot be proved by selecting one out of the several types of pebbles occurring in the conglomerate and showing its resemblance to the matrix. To consider all the other varieties of pebbles which do not conform with the matrix, as xenoliths or segregations is, also, in the writer's opinion, not quite a valid argument. According to Slater, the matrix is a crushed quartz porphyry (H. K. Slater, 1906, p. 4). Sampat Iyengar thought it was the Tarikere gneissic granite (Peninsular gneiss) (1917, p. 113), but later referred it to the Champion gneiss (1920, pp. 7, 9). The writer finds that the matrix is not quite uniform, though there is a general resemblance. The matrix of the lower division is somewhat more basic because of its probable derivation from the spilites and epidiorites ; this grades upwards into a more acidic type with plenty of quartz and grains of felspar. Chlorite forms one of the chief minerals in the matrix of these conglomerates.

Distribution of rutile.—A very suggestive difference between the pebbles and the matrix is seen in the distribution of rutile. Yellow grains of this mineral are abundant in the matrix but are decidedly of sporadic occurrence in most of the pebbles, except in those belonging to spilites and keratophyres. Rutile is more prevalent in the lower division than in the upper, and is authigenic.

Veins in pebbles.—Veins occurring in the pebbles stop abruptly at their boundary with the matrix. This is very well seen both in the field as well as in microsections. It may be mentioned here, that one of the arguments advanced by Wagner for considering certain conglomerates of South Africa as non-sedimentary in origin, is that the original black or brownish-red banding of the chert can sometimes be traced across the intervening matrix from one "pebble" to the other (P. A. Wagner, 1927, p. 55). The matrix of the Kaldurga conglomerate is often schistose as the result of pressure, but there is no parallelism or relationship between this schistosity and the banding of the quartzite or gneiss pebbles ; they are arranged anyhow.

Alternation of pebbly and non-pebbly bands.- Almost throughout the Kaldurga area, pebbly layers are seen to alternate with fine-grained non-pebbly layers. This character imparts a banded nature to the outcrops. The non-pebbly layers are somewhat deeper in colour than the pebbly bands. Slater observed this feature but did not offer any explanation (H. K. Slater, 1906, p. 3). Sampat Iyengar in attempting to explain it by invoking purely igneous phenomena, has suggested that this banding is the "result of an initial segregation into layers of normal, acid and basic portions in the gneissic granite" (P. Sampat Iyengar, 1917, p."108)—a very difficult explanation. This banding can be satisfactorily accounted for by the sedimentary theory, as it is a phenomenon met with in many sedimentary conglomerates. The pebbles are not supplied uniformly, and at certain stages only finer material was deposited. That there is no other difference between these bands, could be seen under the microscope, for the material composing the fine-grained bands is identical with the matrix cementing the pebbles in the conglomeratic bands.

Graded bedding, Current bedding.-- As has been mentioned earlier, the pebbly grits exhibit very good grading on the hills about a mile west of Baliganur. Layers of rocks varying in thickness from 9″ to 15″ occur here, and each layer grades from coarse pebbles at the bottom to fine grit at the top. In other outcrops of these grits, a crude cross bedding also showing grading of the grains is sometimes seen.

Pebbles flattened at right angles to bedding planes.—The pebbles have generally been crushed and flattened. In some cases, the pressure has acted almost parallel to the bedding planes and hence the pebbles appear to stand vertically to the plane of bedding. This can be observed very well on the hills west of Baliganur.

Absence of pebbles of age posterior to conglomerates.— Hill, who was one of the earlier British geologists who recognised the occurrence of crush-conglomerates, states, "in doubtful cases, the only safe test of original deposition

is the presence of foreign fragments sufficiently large to escape being confounded with the materials which form the matrix. Similarly, the most satisfactory proof of deformational structure is the inclusion of igneous boulders derived from rocks posterior in age to the matrix" (J. B. Hill, 1901, p. 327). The only rocks which are distinctly of later age in this area are the granites of the Tarikere valley and those occurring near Kadur and Birur. These granites contain orthoclase and microcline and it is significant that the granite pebbles occurring in the Kaldurga conglomerates do not bear these felspars. It is interesting to note that the granite pebbles occurring in the conglomerates of Lomagundi in Southern Rhodesia, differ precisely in the same way from the intrusive granite of the area (Mennel, 1910, p. 359).

It has already been mentioned that pebbles of banded ferruginous quartzites occur in the conglomerates. Examination of parts of the Shimoga schist belt has convinced the writer, that the banded quartzites were not deposited at one particular time, but that there were varying intervals between the deposition of the several bands. In some cases they are practically pure quartzites showing faint traces of banding. Near Allampur, north of Chikmagalur, the quartzites are current bedded, and this character would, perhaps, have escaped observation, if the successive bands were not demarcated by thin ferruginous layers. Some banded quartzites contain more iron and these grade into the typical ferruginous quartzites with a high percentage of iron. In the explanation of the structure of the area offered by the writer, the banded ferruginous quartzites are seen to be distinctly older than the conglomerates, and hence their occurrence as pebbles is naturally to be expected.

The presence of pebbles of vein quartz and the runs of vein quartz occurring in the area, have caused some confusion, but it must be recognised that these are not all of the same age. The veins connected with the intrusion of the later granites are, of course, later than the conglomerates, and they do not show any signs of pebble formation.

Evidence of the grits.—The assemblage of varied types of rocks occurring as pebbles in hand specimens of the conglomerates has been already mentioned. This phenomenon is more prominently noticed in some of the grits. The small fragments making up the grits are often of different petrographic types. In sections cut from one hand specimen, the writer has recognised fragments of granite, pegmatite, micropegmatite, keratophyre, quartz grains and quartzites of different grain sizes. Several of these are often seen within the compass of a single microsection. The boundaries of these fragments are quite distinct. It would be extremely difficult to explain this intimate mixture of various types of rock by considering that they are crushed xenoliths. There is the interesting possibility that these grits might represent

metamorphosed volcanic tuffs, but till more definite proof of this is obtained, the writer would like to suggest that such a collection of different types of fragments could not have been caused by the action of mere pressure alone. Deposition under water is indicated.

The evidences of pressure on the conglomerates are many, and these are mainly responsible for their having been considered as examples of crush conglomerates. The rock has often developed a schistose structure and the pebbles have been rolled out, elongated or converted into lenticular shapes; sometimes they have been flattened. The effects of pressure are well seen in microsections. Quartz grains usually exhibit undulose extinction, and larger grains frequently show peripheral granulation. Twinning lamellations have been enhanced in plagioclase, and are often broken and faulted.

The writer is not unaware of the possibility that in certain localities in the Mysore State, the effects of pressure and igneous intrusion have been such as to form brecciation of rocks leading to the development of autoclastic conglomerates similar to those described from the Isle of Man (G. W. Lamplugh, 1903, pp. 55-58) and Spitzbergen (G. W. Tyrrell, 1924, pp. 463-64). The evidences put forward in this paper are confined purely to the region under investigation and point definitely to the conclusion that the Kaldurga conglomerates have had a sedimentary origin.

9. Other Ancient Conglomerates in India.

A brief mention may be made here of similar ancient conglomerates recorded from other parts of India. Bruce Foote who considered all the Mysore conglomerates as sedimentary, was of the same opinion regarding those found in the Lower Transitions of the Bellary District, Madras (R. Bruce Foote, 1896, pp. 80, 87, 105-107, 149). Typical crush conglomerates have been described by Hayden from the Lower Haimantas in the valley of the Lipak River (H. Hayden, 1904, pp. 11-12). Maclaren has considered as sedimentary those conglomerates occurring in the Tungabhadra region where pebbles and boulders of granite, apatite, quartz-porphyry, quartzite and banded jasperoid quartz occur embedded in a schistose felspathic matrix containing chlorite (J. M. Maclaren, 1906, p. 108). Underlying the manganese ore band at Ukua and Balaghat, Fermor noticed a rock which appeared to be an ordinary mica-gneiss. Since there were pebbles of various materials like white quartz, granite and gneiss, set in a matrix which resembles the composition of a micaceous gneiss, he considered this rock as a metamorphosed conglomeratic grit, similar to those described by Cunningham Craig from the Loch Lomond district in Scotland (L. L. Fermor, 1909, p. 311). At Rewasa, in Rajputana, sedimentary conglomerates of the Aravalli system contain pebbles of white quartz, pale and dark grey quartzite, white grit and mica

schist ; these are found in a schistose matrix of biotite and chlorite with octahedra of magnetite (A. M. Heron, 1917, p. 17). In north Singbhum, interbedded with phyllitic slates, there are conglomerates which contain pebbles of quartz, banded and normal quartzite, chlorite schist, tourmaline-quartz rock and granite, set in a sericite-quartz schist matrix. The rocks are highly sheared but according to Dr. Dunn they are not autoclastic but sedimentary rocks deposited during periods of intervolcanic erosion (J. A. Dunn, 1929, p. 35). Recently a detailed study of some conglomerates of Dharwar age from Chota Nagpur and Jubbulpore has been made by Dr. Krishnan. The pebbles are of quartzite, micaceous quartz-schist, translucent vitreous quartz, fine-grained biotite schist, phyllite, granite and occasionally tour-maline-quartz rock. The groundmass contains chlorite, sericitic matter, some biotite and magnetite. These conglomerates are considered by Krishnan to be of sedimentary origin, though locally the crushing and shearing have been so intense as to impose autoclastic characters (M. S. Krishnan, 1934, pp. 455-63). A similar conclusion has been arrived at by Ray regarding some of the conglomerates found in the Jubbulpore District (S. K. Ray, 1932, pp. 113-18).

10. Summary.

A very well-developed bed of conglomerate outcrops in and around Kal-durga in the Kadur District. The official opinion of the Mysore Geological Department regarding these conglomerates is, that they are of autoclastic origin. As the result of a detailed study of the pebbles and the matrix, the writer has come to the conclusion that the conglomerates are not autoclastic, but that they are sedimentary formed. The intense crushing and shearing to which they have been later subjected, have tended to obscure their original nature. A very varied assemblage of pebbles has been discovered by the writer. The pebbles are composed of granite, gneiss, pegmatite, aplite, microgranite, granodiorite, granophyre, amphibolite, hornblende schist, serpentine, quartz felspar porphyry, felsite, rhyolite, keratophyre, albite dolerite, spilite, quart-zites, quartz schist, magnetite quartzite, phyllite and limestone. The shape of the pebbles, the mineralogical and textural differences between the pebbles and the matrix, and absence of pebbles belonging to rocks posterior in age to the matrix of the conglomerates are some of the reasons adduced in favour of a sedimentary origin for these conglomerates.

11. Acknowledgments.

The writer is very greatly indebted to Dr. G. W. Tyrrell, D.SC., F.R.S.E., and Professor E. B. Bailey, F.R.S., for help and suggestions during the course of this investigation in the geological laboratory of the University of Glasgow. Thanks are due to Mr. A. M. Sen, M.SC., F.G.S., Director of Geology in Mysore,

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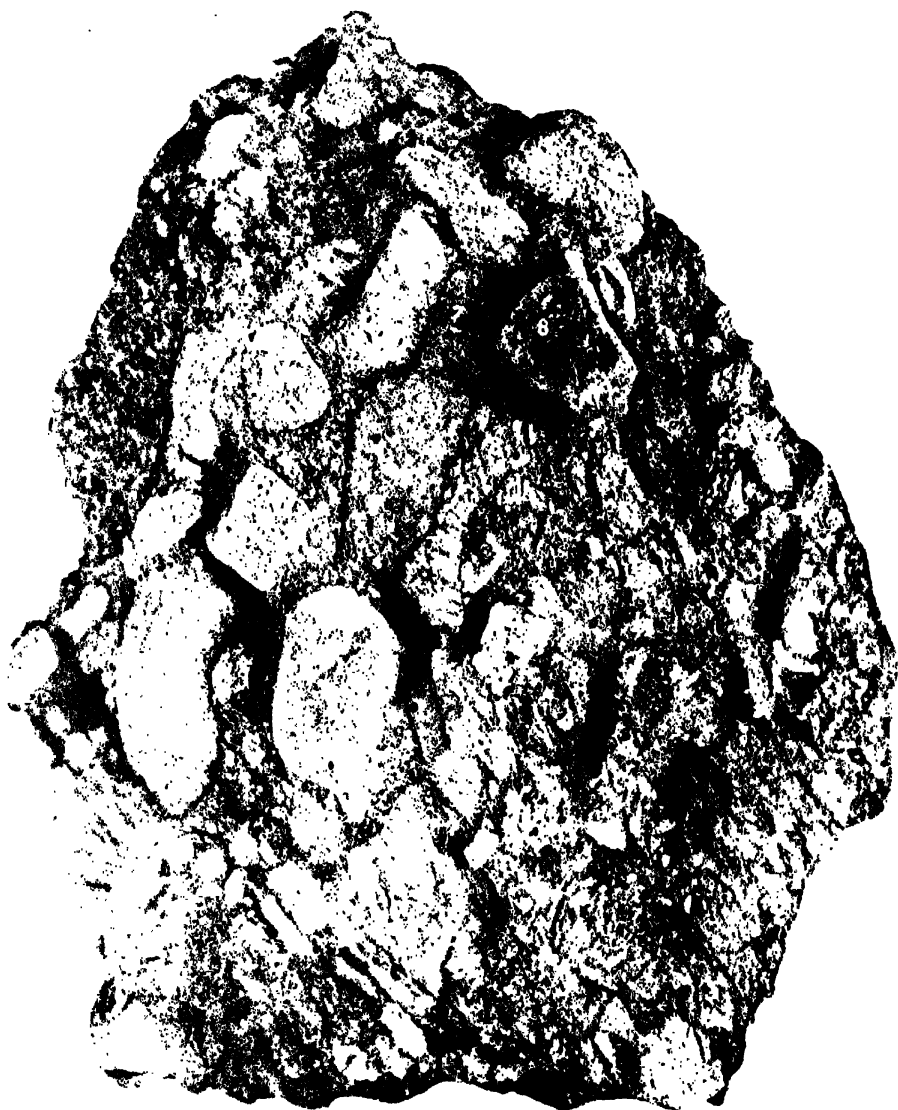
Handspecimen of conglomerate containing eight different kinds of pebbles. 1. Granite. 2. Gneiss. 3. Microgranite. 4. Pegmatite. 5. Quartz Felspar Porphyry. 6. Spilite. 7. Quartz Schist. 8. Quartzite.
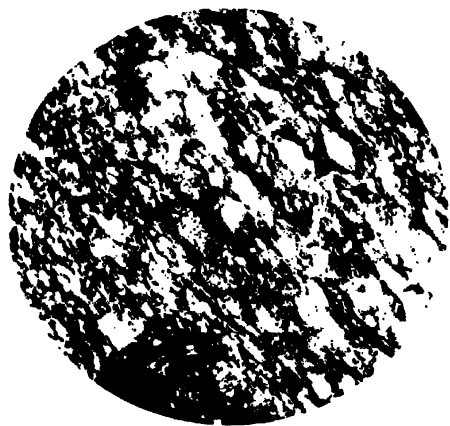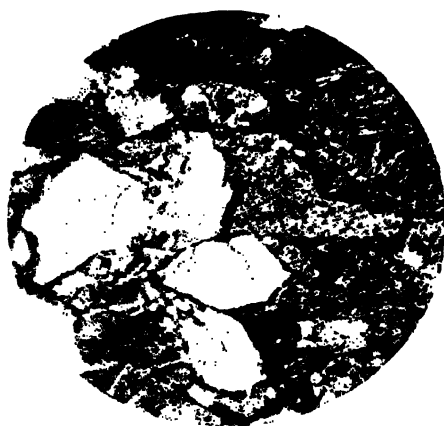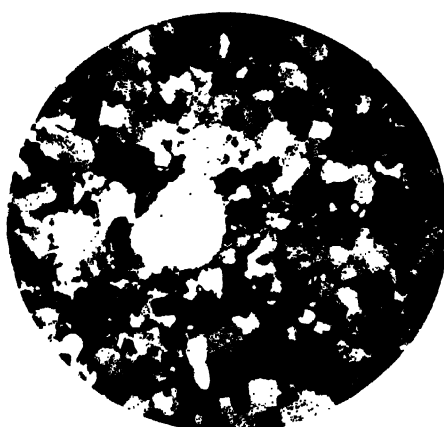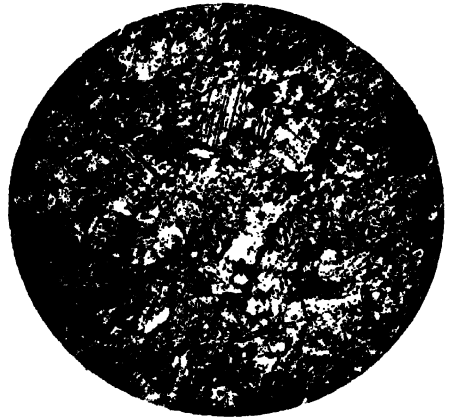
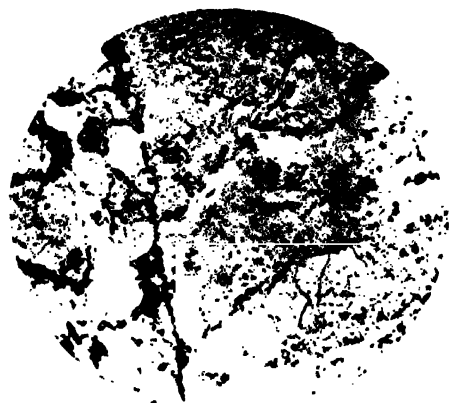FIG. 1.

FIG. 2.

FIG. 3.

Fig. 1.

Fig. 2.

Fig. 3.

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EXPLANATION OF PLATES.

PLATE XXIII.

Specimen of conglomerate containing several types of pebbles, such as granite, gneiss, micro-granite, pegmatite, quartz felspar porphyry, spilite, quartz schist and quartzites. Many of the pebbles are rounded. $\times$ ¾ natural size.

PLATE XXIV.

FIG. 1.—Ordinary light. Sheared chloritic grit. The colourless areas are formed of quartz and the dark portions, mostly of chlorite. $\times$ 22. •

FIG. 2.—Ordinary light. Grit, 1 mile W. of Baliganur. A fragment of somewhat altered spilite is present. The colourless fragments are of vein quartz and quartzite. $\times$ 22.

FIG. 3.—Crossed nicols. Section of granophyre pebble. A crystal of plagioclase is surrounded by the characteristic micrographic intergrowth. $\times$ 30.

FIG. 4.—Crossed nicols. Section of pebble of quartz felspar porphyry. The felspars are almost all of them albites. The groundmass is minutely crystalline. Quartz occurs in patches with a mosaic texture. $\times$ 22.

FIG. 5.—Crossed nicols. Section of quartzite pebble with typical mosaic texture. $\times$ 22.

PLATE XXV.

FIG. 1.—Ordinary light. Section of magnetite quartzite pebble. It is not so ferruginous as the typical hematite and magnetite quartzites of the Bababudans. The banding is emphasised by the parallel arrangement of the minute octahedra of magnetite. $\times$ 22.

FIG. 2.—Ordinary light. Section of limestone pebble. Chlorite and magnetite are associated. $\times$ 22.

FIG. 3.—Ordinary light. A portion of the matrix. The abundant colourless mineral is muscovite, which is associated with chlorite. $\times$ 22.

FIG. 4.—Crossed nicols. Illustrates the sharp contact of a granite pebble with the fine-grained quartzose matrix. $\times$ 22.

FIG. 5.—Ordinary light. The dark thin line separating the pebble from the matrix is composed of chlorite. $\times$ 22.

THE RESPIRATORY SYSTEM AND THE MODE OF RESPIRATION OF THE WATER-BUG, *SPHÆRODEMA RUSTICUM* FABR., WITH REMARKS ON THOSE OF *NEPA*, *LACCOTREPHES* AND *RANATRA*.

By M. J. Presswalla
AND
C. J. George.
(*From the Department of Biology, Wilson College, Bombay.*)

I. *Introduction and Historical Survey.*

ALL adult insects are air breathers, and whether they live in air or water they depend upon atmospheric air for their respiration. The immature stages of a few insects have become completely independent of atmospheric air by developing blood gills or tracheal gills for respiring dissolved oxygen from the water, while most are like the adult air breathers. This is so because insects are primarily terrestrial creatures and have, like the aquatic mammals, taken to water secondarily, possibly on account of keen competition on land. Amongst the adult insects a few of the Heteroptera and Coleoptera alone are perfectly adapted to live in water. These water-bugs and water-beetles carry a supply of air under their wing-covers and swim about freely in water, their hind legs acting as efficient oars. While submerged they breathe this air by means of the dorsal thoracic spiracles. Occasionally they come up to the surface to renew this supply. When on the surface, they breathe directly from the atmosphere by means of the last pair of abdominal spiracles which is specially adapted for the purpose.

Of the Heteroptera, the Cryptocerata are aquatic or semi-aquatic. The aquatic families of the Cryptocerata are: Naucoridæ, Belostomatidæ, Nepidæ, Notonectidæ, Pleidæ and Corixidæ,—all of these have peculiar adaptations which have solved the problem of breathing the atmospheric air under water. The study of these insects has occupied the attention of various workers, but except for *Nepa* and *Ranatra* of the family Nepidæ, detailed work has not so far been done on these insects. Of the earlier investigators, Dufour made a wide study of the aquatic Hemiptera. He was the first (1821) to note the "tracheo-parenchymatous" organs peculiar to the non-flying aquatic bugs, in *Nepa* and *Ranatra*. Martin (1896) pointed

out certai : respiratory adaptations in the nymphs of the Belostomatidæ, Naucoridæ and Nepidæ. Dogs (1908), Maulik (1916) and Brocher (1916) studied the respiratory system of *Nepa cinerea* in detail. Ferrière (1914) made a comparative study of the "tracheo-parenchymatous" organs of *Nepa*, *Ranatra* and *Naucoris*. Muttkowski (1920) gave a collective review of the respiration in the aquatic insects. In 1921 Muttkowski made a valuable contribution on the rôle of insect blood in carrying oxygen. This function of insect blood has thrown a new light on the study of the respiration of aquatic insects. Poisson (1924) in his study of the aquatic Hemiptera dealt with the "tracheo-parenchymatous" organs, especially of *Nepa*. Recently, Lee (1929b) and Wigglesworth (1931) reviewed the subject of the respiration of insects. Hamilton (1931) in her monograph on *Nepa cinerea* added to our knowledge of the respiratory system of this insect. The above records show that except for *Nepa* detailed studies on the respiration of other aquatic insects have not been undertaken, and there is a valuable field awaiting investigation.

Sphærodema rusticum Fabr. which forms the subject of this paper belongs to the family Belostomatidæ. Apparently no work has been done on this insect, and while studying its respiratory system, the writers undertook also to work out its morphology. This has however been incorporated in a separate paper. The only record found on this water-bug is by Hoffmann (1926) who studied the life-history of this species, and described the five nymphal instars.

The material for the present study was collected from a fresh-water tank in a suburb of Bombay. Together with *Sphærodema* were also collected specimens of *Laccotrephes elongatus*, *L. grisea* and species of *Ranatra* (Nepidæ), *Plea* (Pleidæ), *Notonecta* (Notonectidæ) and *Microvelia* (Hydrometridæ) and comparative observations made on them. Specimens of *Nepa cinerea* were obtained for comparison of certain structures from the British Museum (Nat. Hist.).

Sphærodema rusticum—The two forms.

Sphærodema rusticum manifests a remarkable case of alary dimorphism. One form possesses normal wings while the other has degenerate wings. In the latter the hemelytra have lost the membrane portion and the inner wings their entire anal region while the remaining portion of the inner wing is very much reduced in size. Intermediate forms with degenerate inner wings but with the hemelytra having the membrane portion partly or fully developed were also found. The differences in the wings have an important bearing on the structure of the thorax. The indirect wing muscles together with the phragma to which they are attached are found missing in the degenerate

winged form, while in the full winged form these parts are present, but the wing muscles have been modified to form the peculiar structures called the "tracheo-parenchymatous" organs. Associated with these organs are a few air sacs in the mesothorax which are also absent in the degenerate winged form. The significance of these differences in the two forms is not quite clear. The degenerate winged form devoid of these structures does not seem to be adversely affected in any way. The general morphological characters and those of the genitalia of the two agree and establish that they are two forms of the same species. The presence of intermediate forms and a common habitat also suggest that they are conspecific.

Laccotrephes and *Ranatra*, collected along with *Sphærodema*, invariably showed normal winged forms only, although these have also lost the power of flight and have their wing muscles modified into the "tracheo-parenchymatous" organs.

II. The Respiratory System of the Adult.

1. The Tracheal System (Fig. 1).

There are two main longitudinal tracheal trunks (A.T.) running along the dorsal region of the abdomen. Posteriorly they open on the ventral wall of two strap-shaped retractile organs (R.O.), which represent the modified eighth abdominal segment, by a pair of spiracles (8th Abd. Sp.). Anteriorly each trunk terminates by a very large spiracle (1st Abd. Sp.) situated dorsally on the intersegmental membrane between the metanotum and the first abdominal segment, on the margin of the body. A very stout trachea, the thoracic spiracular trunk (S.T.), connects this large spiracle with a spiracle (Mt. Sp.) on the dorsal anterior side of the metathorax, below the base of the wing. From this the spiracular trunk is continued into the prothorax obliquely inwards, as the main thoracic trunk (T.T.). The trunks of the two sides converge into the head. In the anterior part of the prothorax each of these trunks bifurcates into a dorsal and a ventral branch, the former (H.T.) supplying tracheoles to the head and the brain, and the latter (R.T.) leading into the rostrum. Immediately on entering the prothorax, on each of these oblique thoracic trunks opens a large oval spiracle (Ms. Sp.). This spiracle is situated on the ventral wall, attached to the intersegmental membrane between the pro- and meso-sterna.

From each of the two abdominal trunks (A.T.) are given off to the outside six lateral abdominal branches (S.B.) in segments first, third, fourth, fifth, sixth and seventh, each terminating in a spiracle on the sternal wall. The first abdominal spiracular branch is very short and lies in the reduced first abdominal segment. This branch supplies a cluster of tracheæ to the
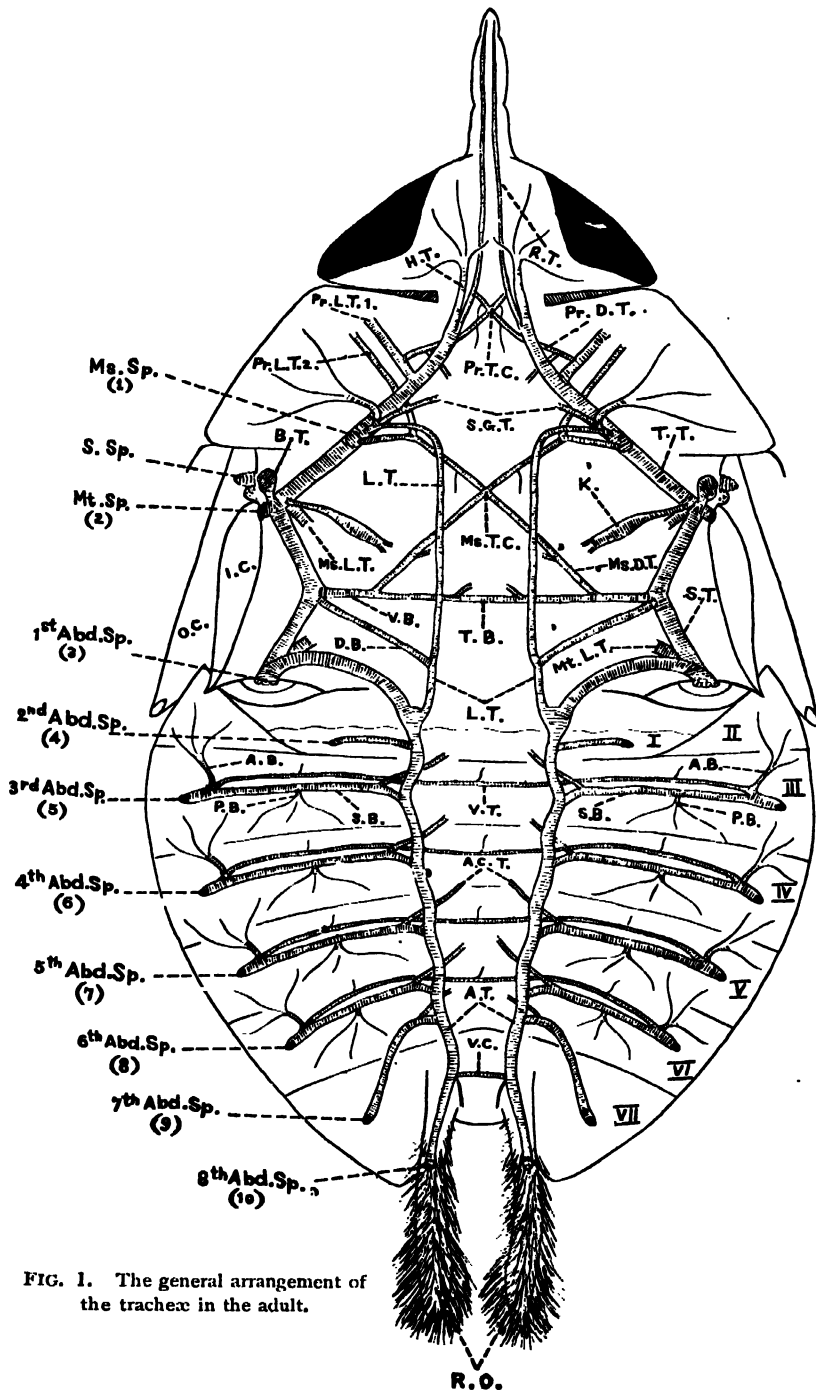
FIG. 1. The general arrangement of
the tracheæ in the adult.

reproductive organs in both the male and the female. The second abdominal segment being very much reduced and completely overlapped by the first it possesses no spiracular tracheal branch and no spiracle. The remaining five lateral abdominal branches lie in segments three to seven and end in spiracles on the ventral wall of these segments. The spiracular branches of the abdominal segments, three to six, each gives out three secondary tracheæ. One (A.C.T.) arising anteriorly near the inner end supplies the digestive organs. A second (P.B.) originates posteriorly from the middle of the spiracular branch and supplies the fat body and the body wall of the surrounding region. The third branch (V.T.) is again anterior and emerges nearer the spiracle. It immediately runs ventrally inwards and joins with the corresponding trachea of the opposite side. It sends out tracheoles to the ventral body wall of its segment. At its origin, an outer anterior branch (A.B.) supplies the outer connexival region between the spiracle of its own segment and that of the preceding one. The lateral abdominal spiracular trachea of the seventh segment gives only one branch which ramifies into the hind gut. Behind this region, the main longitudinal trunk, before it terminates in the eighth abdominal spiracle, gives out on the inside, fine branches to the tip of the abdomen and the genitalia. Also, a fine ventral transverse commissure connects the two main trunks in the seventh segment (V.C.).

At the point where the main abdominal trunks (A.T.) diverge into the metathorax, emerges a pair of longitudinal tracheæ (L.T.) which runs parallel in the mid dorsal region of the thorax and terminates dorsally on the main thoracic tracheæ (T.T.) of the respective sides, just above the large oval spiracles (Ms. Sp.). These longitudinal tracheæ are very thin in the degenerate winged form but in the full winged form they are stout and pass through the dorso-longitudinal muscles which form the " tracheo-parenchymatous" organs, giving out numerous transverse branches on all sides. The longitudinal tracheæ of the thorax before they terminate on the main thoracic tracheæ, give out branches to the salivary glands (S.G.T.) and to the dorsal outer body wall of the prothorax. From the middle of the thoracic spiracular trunk (S.T.) arise two transverse branches; one of these, the dorsal branch (D.B.), joins the longitudinal trachea (L.T.) of its side as that trachea enters the scutellar region, and in the normal winged form supplies tracheæ to the dorso-lateral muscles which form the subsidiary " tracheo-parenchymatous" organs; the other, the ventral branch (V.B.), divides into two; the posterior of these two offshoots (T.B.) runs transversely and joins the corresponding branch of the opposite side, while the anterior one (Ms. D. T.) runs diagonally forwards and terminates dorsally on the main thoracic trachea

of the opposite side. As the diagonal branches of the two sides cross in the middle of the mesothorax they unite with each other to form a big ventral tracheal cross (Ms.T.C.). This cross supports the mesothoracic nerve ganglion and supplies tracheoles to it. Just before its anterior termination the diagonal trachea (Ms.D.T.) gives off a thin branch (Pr. L.T. 2) to the prothoracic leg. Another stouter branch (Pr. L.T. 1) from the main thoracic trachea (T.T.) also supplies the prothoracic leg. At the junction where the main thoracic trachea and the spiracular trunk trachea meet, is given out a short club-like tracheal branch (K) which lies transversely on the dorsal side, in the mesothorax. It ramifies into the surrounding tissue. From the junction just referred to arises on the outer margin the short but stout metathoracic spiracular branch. This branch gives out anteriorly a short open bulbous trachea (B.T.) which lies freely in a membranous cavity of the body wall. This bulbous trachea being open and unattached distally, air from it passes into the body cavity. From the first abdominal spiracle an independent stout trachea (Mt. L.T.) to the metathoracic leg and from the mesothoracic spiracular branch a stout trachea (Ms. L.T.) to the mesothoracic leg are supplied. Of the two prothoracic leg tracheæ, the stouter one (Pr. L.T.1.), before it enters the leg, gives out a ventral diagonal branch (Pr.D.T.) which joins the rostral branch (R.T.) of the opposite side. The two diagonal tracheæ of the opposite sides as they cross each other unite to form in the prothorax a small tracheal cross (Pr.T.C.) similar to the one found in the mesothorax. This cross supports the subœsophageal ganglion and supplies tracheoles to it.

2. *The Tracheo-parenchymatous Organs and Air Sacs* (Fig. 2).

The respiratory systems of the two forms of adults are similar in all respects except for certain air sacs, the so-called " tracheo-parenchymatous organs " and a pair of chitinous phragma connected with their attachment, which are present only in the full winged form. When the scutellum of the normal winged adult is removed, the " tracheo-parenchymatous organs " (D.T.O. and S.T.O.) become visible. These organs have been the subject of intensive study in *Nepa, Ranatra,* etc., by various investigators, notable amongst whom is Ferrière who devoted an entire paper to the comparative study of these organs in *Nepa, Ranatra* and *Naucoris.* In *Sphærodema* these organs consist of two pairs, a dorsal longitudinal pair and an oblique lateral pair. The dorsal organs (D.T.O.) are attached anteriorly to a semi-circular chitinous apodeme, an endotergite from the anterior margin of the scutellum, and posteriorly to a broad V-shaped phragma projecting down from the posterior margin of the metanotum, which lies below and a little anterior to the apex of the scutellum. These two endotergites are special
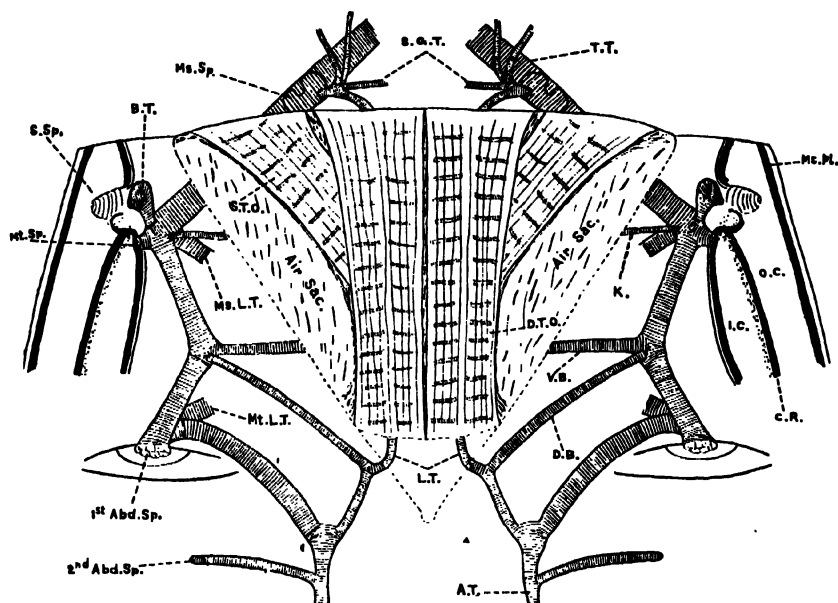
Fig. 2. The tracheation of the thorax--Macropterous adult.
(Showing the tracheo-parenchymatous organs and air sacs.)

developments for the attachment of these organs, and together with these organs are found missing in the degenerate winged form. The oblique lateral organs are attached anteriorly to the curved down anterior margin of the scutellum outside the attachment of the dorsal longitudinal organs. These, known as subsidiary "tracheo-parenchymatous organs" (S.T.O.) run backwards and inwards and going below the dorsal organs attach themselves posteriorly to two thin apodemes from the metanotum. These thin apodemes are present in the degenerate winged form also, although in that form no muscles are attached to them. "

The "tracheo-parenchymatous organs" are made up of a network of tracheæ enclosing thin longitudinal parenchymatous cells. The tracheation of the organs can best be seen by soaking them in a 10% solution of Caustic Potash for over forty-eight hours. This leaves the tracheal network free of the fibres. The histology of the organs can be better understood from microtome sections. The dorsal longitudinal tracheæ of the thorax (L.T.) pass through the dorsal organs giving out numerous transverse branches on all sides which ramify in the parenchymatous tissue. These organs have been identified with the dorso-longitudinal indirect wing muscles by Ferrière and others. The subsidiary organs are supplied with tracheæ from the branch D.B. Longitudinal sections (Figs. 4 and 5) reveal the muscular

nature of the organs. The fibres are obviously muscle fibres and bear large prominent nuclei, but they however show no cross striations of the normal muscle fibres. From a comparison of these organs with the indirect wing muscles of flying insects it is evident that there are fewer muscle fibres and more tracheal branches in these organs. The tracheæ themselves are more inflated than in the ordinary muscles. The openings of the transverse branches into the longitudinal central trachea, are relatively smaller in diameter than the branches themselves (Fig. 4). Ferrière lays stress on this point suggesting it as a means of retaining air in the transverse branches and preventing it from getting back into the central trachea.
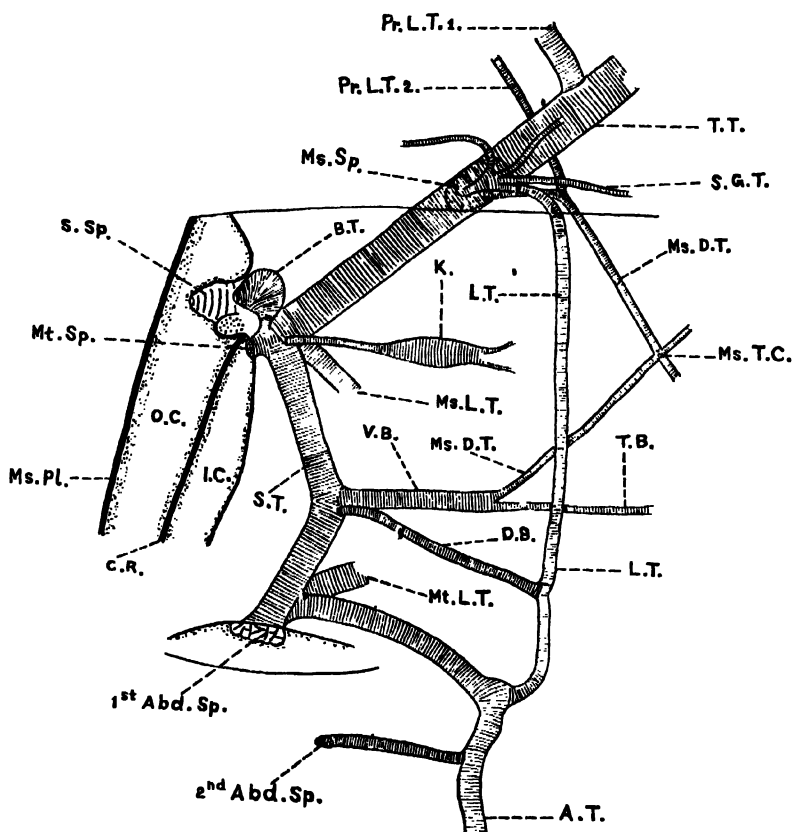

FIG. 3. The tracheation of the thorax—Degenerate winged adult.

In the degenerate winged form the longitudinal thoracic tracheæ (L.T.) are very thin and bear no transverse branches along their lengths. They lie loosely suspended in the thorax (Fig. 3).

Dufour (1821) was the first to describe the tracheo-parenchymatous organs of *Nepa* and *Ranatra*. He and Dogs (1908) assign to them a pulmonary function. Ferrière (1914), Brocher (1916), and Poisson (1924) regarded them as degenerate indirect wing muscles with no apparent function, being vestigial organs. Ferrière believed that the tracheation of these organs is the normal tracheal supply of the wing muscles, but this view can hardly be accepted, since in comparison with the normal wing muscles of insects like the cockroach and the honey-bee these organs have more and larger tracheæ and fewer muscle fibres as already stated. Transverse sections of
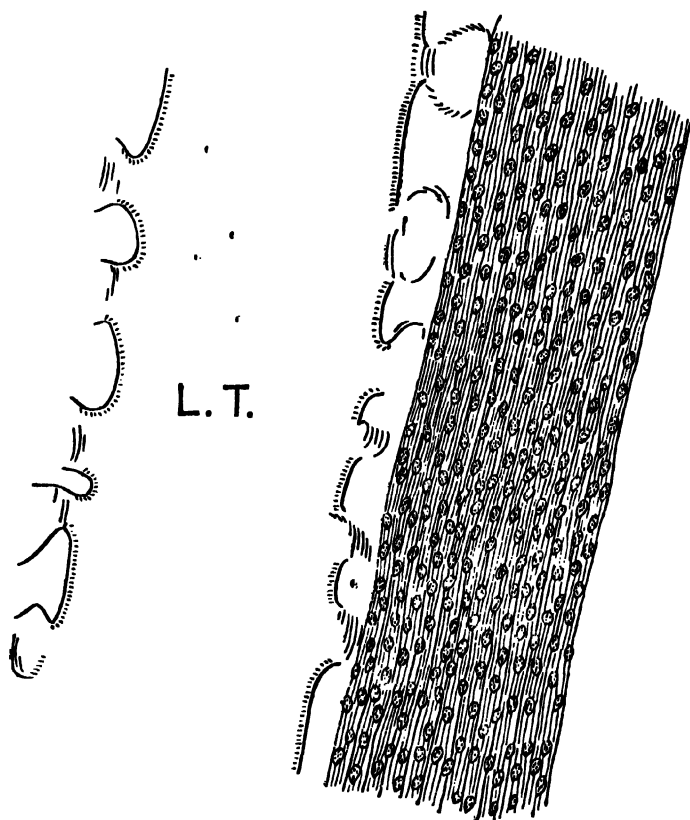

L. T.

FIG. 4.   L. S. through the centre of the tracheo-parenchymatous organ. × 366·67

these organs (Pls. XXVI and XXVII) show that the structure is not that of a normal muscle. Maulik (1916) suggested that these organs served to store air. Poisson considered the degeneration of the muscle fibres as due to phagocytosis, but examination of the fibres in *Sphærodema* has not shown any phagocytes in them. Hamilton (1931) discusses the various viewpoints

in her paper on *Nepa*, and she also disagrees with Ferrière as regards the tracheation of these organs and with Poisson as regards the presence of phagocytes. She is however of opinion that these organs cannot be without any function as Ferrière and others suggested, although their histology has so far failed to indicate anything definitely. She concludes that physiological investigations only may throw some light on the matter, but she is doubtful about the possibility of devising any satisfactory experiment for the purpose.

In addition to these organs the full winged adult possesses a pair of large air sacs (Fig. 2) on the dorsal side and a number of smaller ones on the ventral side in the mesothorax. The functions of the air sacs, which are best developed in actively flying insects, like the honey-bee, have been variously interpreted. Snodgrass (1925) says : "The reason for the air sacs of insects is a subject concerning which there has been considerable difference of opinion" (p. 203). Some think that they give buoyancy to the insect in flight. But this has been disputed from the physical point of view. Others suggest that they store air for the respiration of the insect when actively flying, as breathing through the spiracles would then be difficult. But insects cannot exist for any length of time without taking in atmospheric air through the spiracles. Lee (1929*a*) gives a very feasible explanation
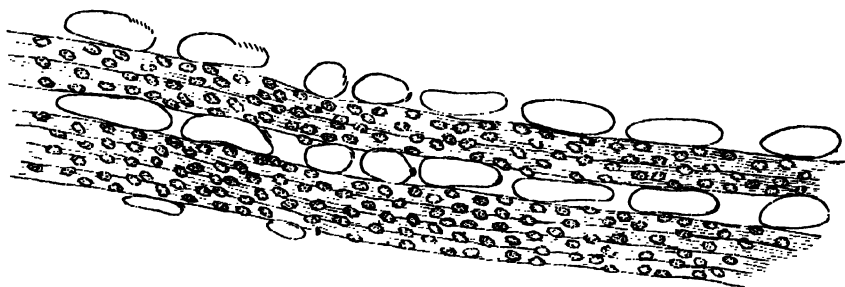

FIG. 5. I.S. through the periphery of the tracheo-parenchymatous organ. × 366·67

of the presence of air sacs in insects. He considered their function as " chiefly mechanical, in allowing a considerable volume of air to be inhaled and exhaled, thereby causing greater ventilation of the larger tracheal trunks than would be possible otherwise".

As *Sphærodema* shows two forms, one possessing these organs and sacs and the other not, it was possible to devise some experiments to see the nature of the function of the tracheo-parenchymatous organs. Specimens of both these forms were kept submerged in water and were prevented by

wire-netting from reaching the surface for a renewal of air. The individuals of both forms died or became stupefied at almost the same time. These results show that the possession of the "tracheo-parenchymatous organs" and air sacs gives no respiratory advantage to the full winged form.

The specific gravities of the two forms were then estimated to see if the possession of these organs and sacs made any appreciable difference in the buoyancy of the two. Both were found to be lighter than water, but the degenerate winged form was found to be denser than the full winged form. The specific gravity of the former was ·92, and that of the latter ·88. Granting that other structures are similar in the two forms, it has to be concluded that the full winged form is more buoyant, most probably on account of the presence of air sacs and the "tracheo-parenchymatous organs". The conclusion therefrom is that the "tracheo-parenchymatous organs" have no apparent respiratory function but they in conjunction with the air sacs or otherwise, decrease the specific gravity of the animal. This feature may be advantageous for the insect in coming up to the surface of the water but it is a distinct disadvantage in going down.

We are of opinion that the "tracheo-parenchymatous organs" are only an intermediate stage in the degeneration of the indirect wing muscles which have completely disappeared in the other form, and have no apparent function. This view also gains support from Ferrière who noted different grades in the degeneration of these muscles in *Nepa* and recorded four individuals of that insect in which he found the wing muscles perfectly well developed. Poisson recorded 5 specimens of *Nepa*, out of 300 that he examined, having "des muscles vibrateurs normeaux". Such individuals may therefore be capable of flight. Three specimens of *Sphærodema* were collected in flight and an examination of their indirect wing muscles revealed normal structures. Brocher from his study of *Nepa* came to the conclusion that the aquatic Hemiptera are on the road to lose their power of flight. "Le fait est d'autant plus interessant que les Hémiptères aquatiques sont effectivement en train de perdre la faculté de voler. Chez plusieurs d'entre eux, il existe déjà des formes aptères ; par example chez la Naucore et chez l'Aphelocheirus." He is of opinion that evolution in these insects takes the line of developing wingless forms and that in course of time *Nepa* also will evolve apterous forms like the apterous Naucoridæ. "Nous saisissons donc, pour ainsi dire, au début, l'evolution vers une nouvelle forme. De même qu'il y a des Naucores ailées et des Naucores aptères, il apparaîtra peut-être, plus tard, des Nèpes aptères."

3. *The Spiracles* (Fig. 1).

All Rhynchota, without exception, possess ten pairs of spiracles, three on the thorax and seven on the abdomen. In naming the thoracic spiracles of Rhynchota, Schiödte used the terms pro-, meso- and meta-thoracic spiracles respectively, because of their situation and because they supply tracheæ to these segments. Maulik followed him in describing the thoracic spiracles of *Nepa*. Recent embryological studies, however, show that the prothoracic spiracle of the embryo is lost in the Rhynchota, as in many other insects, and that the three pairs of spiracles present in the thorax in the post-embryonic stages, are of the mesothoracic, metathoracic and first abdominal segments respectively. Imms mentions : "Among adult winged insects there is no indubitable instance of the prothoracic spiracles being present. Those often regarded as belonging to this segment pertain in all probability to the mesothorax, having undergone a secondary forward migration." Brocher and Hamilton have taken this view and have called the three thoracic spiracles of *Nepa*, mesothoracic, metathoracic and first abdominal respectively. We have followed this enumeration as it seems to be the more correct.

In *Sphærodema*, the first and the second abdominal segments are very much reduced. Though the first abdominal segment bears a pair of spiracles the second is without any. Since the pair of spiracles on the metathorax is to be regarded as belonging to the first abdominal segment, the spiracles found on the first segment should be regarded as those belonging to the second abdominal segment. The rest of the abdominal segments (3 to 8) each bear a pair of spiracles of its own.

The Mesothoracic Spiracle.—The first spiracle (Ms. Sp.) lies on the membrane between the pro- and the meso-sterna, and is completely hidden
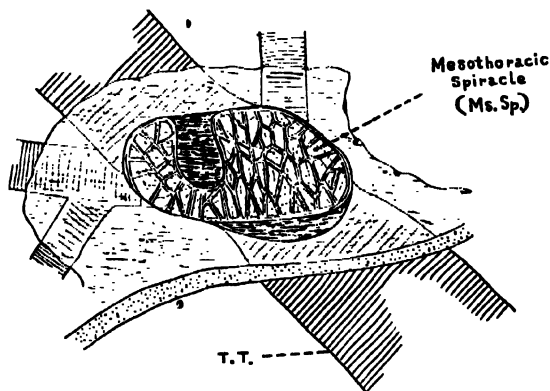

FIG. 6. Mesothoracic spiracle of the adult. X 55·34.

from view. To expose it, it is necessary to remove a portion of the pro-pleuron. The spiracle is very large and oval and is peculiar in being placed not at the end of a trachea but on the wall of the main thoracic trachea just as it enters the prothoracic region. In *Laccotrephes*, *Nepa* and *Ranatra* we found this spiracle in the same position but the fact is generally not made known. Although Schiödte and Bueno described its position correctly in *Nepa* and *Ranatra*, both Maulik and Hamilton showed it in *Nepa* at the end of a trachea. Brocher's figures of this spiracle in *Nepa* give the appearance of being on the wall of a trachea but he does not give any description of its structure.

This spiracle is covered by a thin membrane having a network of chitinous veins (Fig. 6). It opens into a side cavity of the prothorax, formed by the lateral extension of the tergum and sternum of that segment. This air chamber is closed but could be made to open to the outside if the pro-thorax is flexed ventrally. The spiracle therefore though ventrally situated has to get its air connection on the dorsal side.

The Metathoracic Spiracle and the Supernumerary Spiracle.—The second spiracle (Mt. Sp.) is more difficult to locate than the first. It is completely hidden from view by a longitudinal chitinous ridge (C.R., Figs. 2 and 3) arising from the upturned meso-pleuron (Ms. Pl.) which forms an air channel
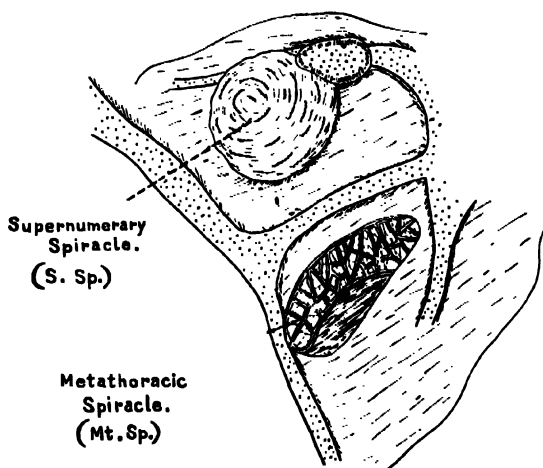

Supernumerary
Spiracle.
(S. Sp.)

Metathoracic
Spiracle.
(Mt. Sp.)

FIG. 7. Metathoracic spiracle of the adult. × 55·34.

on the side of the metathorax. This chitinous ridge divides the air channel referred to above into two, an inner chamber (I.C.) and an outer chamber (O.C.). The ridge also closes completely the inner channel on the dorsal side and so hides the metathoracic spiracle which opens inside. On carefully

removing the chitinous ridge the spiracle is exposed, but even then it is not easily detected being inconspicuous. In structure, it is similar to the mesothoracic spiracle having a membranous covering with a chitinous network (Fig. 7). It is however located at the end of a short tracheal branch unlike the mesothoracic spiracle.

Another peculiar structure (S.Sp.) which is likely to be mistaken for the metathoracic spiracle lies immediately anterior to it and projects into the outer air channel. This structure is a membranous hood-like projection. The membrane is strengthened by fine zig-zag chitinous rings (Fig. 7). It becomes visible on removing the hemelytron, near the base of which it lies. If observed for a time it is seen to expand into the outer channel at every exhaling action of the insect, and relax rhythmically like the action of a bellows. This expansion is due to air being forced into it from inside the body. In describing the tracheal system it was mentioned (p. 10) that from the short metathoracic spiracular trachea arises a short open bulbous trachea (B.T., Figs. 1, 2 and 3). The terminal portion of this bulbous trachea lies loosely in the cavity formed by the membranous hood and opens into it. Air coming out of this bulbous branch escapes into the hood and diffuses out through its membrane. This structure we have termed the Supernumerary Spiracle since it cannot be regarded as belonging to the normal spiracular number. As the bulbous trachea is free distally, part of the air it gives out passes into the body cavity as well. Figs. 3 and 4 give a general view of the whole structure.

It has been described above that the metathoracic spiracle (Mt.Sp.) opens into the inner air channel (I.C.) and the supernumerary spiracle (S.Sp. opens into the outer air channel (O.C.). These air channels are in communication with the storage air under the Hemelytra. The air channel is divided into two distinct chambers probably because there are two kinds of air in them. The structure of the hood is meant only to expand outwardly when air is given out by the bulbous trachea. Observations on it do not show any indication of an inward current passing into this spiracle from the air channel. It is reasonable therefore to assume that the outer channel is an excurrent channel, impure air passing out from the supernumerary spiracle into it, and that the inner channel must be an incurrent channel taking in pure air to the metathoracic spiracle from the air storage under the hemelytra.

Identical arrangements are found in *Belostoma*, *Laccotrephes elongatus*, *L. grisea*, *Nepa cinerea* and *Ranatra*. But neither Maulik (1916) nor Hamilton (1931) has made any reference to the true metathoracic spiracle in their papers on *Nepa*. The figures they give of the metathoracic spiracle (Maulik's mesothoracic) and the descriptions thereof are really of the supernumerary

spiracle. Both these authors therefore have mistaken the supernumerary spiracle for the true metathoracic spiracle, in spite of the fact that its structure is very different from the other spiracles on the thorax. The metathoracic spiracle being small and completely hidden in the inner closed channel has evidently been overlooked and the more prominent supernumerary spiracle lying in the outer channel mistaken for the former.

In *Nepa, Laccotrephes*, etc., there is another structure similar to the supernumerary spiracle near the third (first abdominal) spiracle on the metanotum. This however has no tracheal connection to it. Hamilton has noted this structure in *Nepa* and has called it a " false spiracle " as it has no trachea opening into it. She noted the similarity between her "metathoracic (supernumerary) spiracle" and her "false spiracle" but gave no explanation as to their homologies or their functions. Brocher (1916) observed and described both these structures, the " supernumerary spiracle " and the " false spiracle", in the meso- and meta-thorax of *Nepa* correctly, but he was unable to say what these organs were. He called them simply enigmatical organs. In a note about these he confesses : " J'ignore ce qu'est cet organe énigmatique ; un autre semblable se trouve tout près et un peu avant du premier stigmate abdominal".

We have observed both the supernumerary and the " false " spiracles of *Laccotrephes* expanding slightly at exhalation as does the supernumerary spiracle of *Sphærodema*. The " false " spiracle, though unconnected with any trachea, seems to give out air, probably that which is given out by the bulbous trachea into the body cavity. We presume therefore that both serve the same function, *viz.*, that of giving out air. Neither Brocher nor Hamilton suspected this fact and so they were unable to assign any function to these organs in *Nepa*. In *Sphærodema* and *Belostoma* the so-called false spiracle is not present. The structure of these organs in the two families Belostomatidæ and Nepidæ differs. The membranous covering in *Sphærodema* and *Belostoma* is loose and hood-like and so can expand far out into the air channel. In *Nepa* and *Laccotrephes* the membrane is circular and expands but slightly. The fine chitinous markings in the former are in zig-zag rings, while in the latter they are more or less straight or curved lines.

*The First Abdominal Spiracle.—*The third spiracle (1st Abd. Sp.) is placed between the metanotum and the first tergum of the abdomen, and opens directly into the subelytral air chamber from which it draws its air supply. It is about as large as the first spiracle (Ms.Sp.), and is very prominent and easily spotted on opening the wings. The structure of the membranous covering is similar to that of the first two spiracles on the thorax
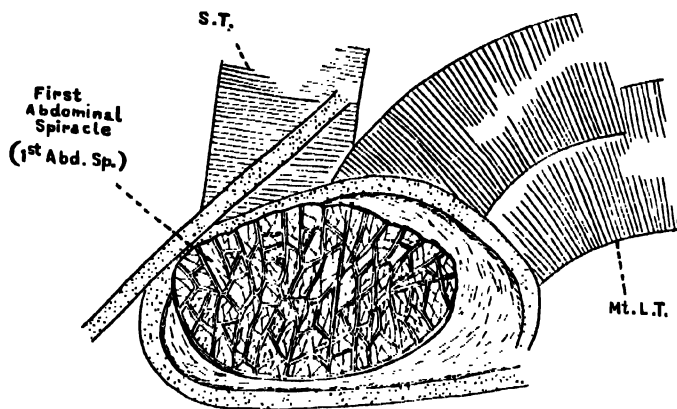
FIG. 8. The first abdominal spiracle of the adult. X 55·34.

but the membrane protrudes a little and is not flush with the surrounding body wall as in the others (Fig. 8).

The Second Abdominal Spiracle.—The fourth spiracle (2nd Abd. Sp., Fig. 1) is again very difficult to find. It is small and lies completely hidden on the reduced membranous first abdominal sternum which is telescoped into the metathorax. The spiracle can be noticed only on separating the abdominal sternite from the thorax. In structure it is similar to the spiracles on the thorax. Being covered over by the metasternum it becomes non-functional though by itself it is not blocked up like the following abdominal spiracles.

The Third to the Seventh Abdominal Spiracles.—The second abdominal segment is very much reduced being pushed back by the projecting metasternum and only a pair of small triangular side pieces remains. The segment therefore bears no spiracle, its spiracle being pushed to the first segment as mentioned above. The third and the following abdominal spiracles therefore lie on their proper segments. The third to the seventh abdominal spiracles (3rd Abd. Sp.—7th Abd. Sp., Fig. 1) or serially the spiracles fifth to ninth, are all ventral, situated on the connexivum of the abdomen. As the ventral side of the abdomen is constantly bathed in water and as there is no means of bringing these spiracles into communication with the external atmosphere, they are completely closed. They are thus rendered functionless and are noticeable only as small round dark spots. In *Nepa* and *Ranatra* the spiracles of the 4th, 5th and 6th segments are said to be modified as abdominal sense organs, but in *Sphærodema* no such modification is found.

The Eighth Abdominal Spiracle.—The tenth and the last pair of spiracles (8th Abd. Sp., Fig. 1) is situated on the ventral side of the base of the

strap-shaped retractile organs situated at the hind end of the abdomen
(R.O., Fig. 1), and together with this is drawn into a cavity of the seventh
abdominal segment, when the insect is under water.   While the insect is on
the surface of the water the retractile organs are pushed out above the surface
film and the spiracles come into play there.   This is the only functional
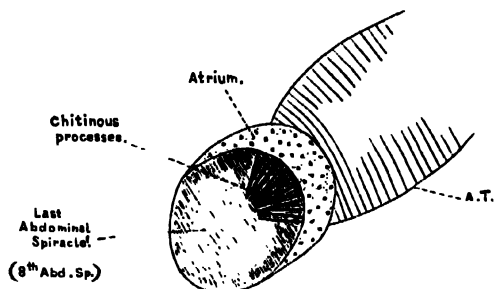pair of spiracles on the abdomen of the adult insect.   The structure of this

FIG. 9.   The eighth abdominal spiracle of the adult   × 150.

spiracle is peculiar ; the opening leads into a cup-shaped atrium inside
which a number of chitinous processes form a sort of cone pointing towards
the opening (Fig. 9).   These chitinous rod-like projections prevent any
particles from getting into the spiracle and choking it.   The long hairs
of the strap-shaped organs are hydrofuge and prevent water from getting
to the spiracle.

4.   *The Retractile Organs*   (Fig. 4 and Pl. XXVIII).

The strap-shaped retractile organs along with the anal and genital
segments lie completely drawn inside the seventh abdominal segment.   At
the base of these organs opens ventrally the last pair of abdominal spiracles
(8th Abd. Sp.) at the posterior end of the main longitudinal tracheal trunks
(Fig. 10).   The retractile organs are developed from the eighth abdominal
segment.   The sides of the eighth segment are drawn out to form these
organs while the rest of the segment appears dorsally as a membranous
bridge and ventrally as two narrow triangular sternites which support the
genitalia.   The strap-shaped organs are thickly covered with long hydrofuge
hairs all directed backwards.   These hairs are longer towards the tips of
the organs.   In the male the tips are more hairy than in the female.

The strap-shaped retractile organs are characteristic of the Belosto-
matidæ.   Bueno and others noted them in *Belostoma*, but their function
has not so far been clearly defined.

When *Sphærodema* comes to the surface of the water to take in atmos-
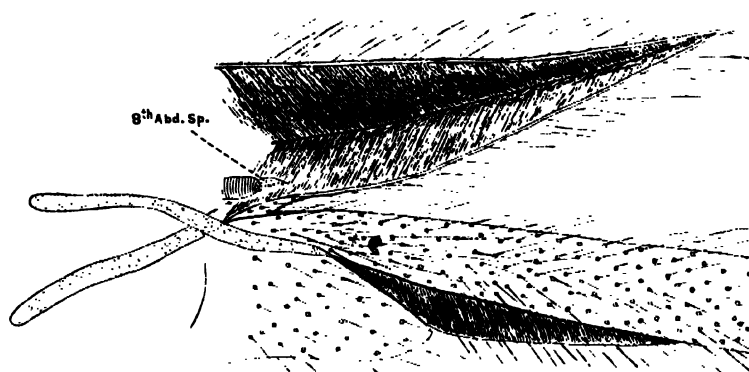pheric air it pushes out the anal organs which pierce the surface film and

FIG. 10. Basal portion of the retractile organs showing the position of the last abdominal spiracle. × 33.

air is led to the last pair of abdominal spiracles through the thick coating of hairs. The atmospheric air is respired in this manner directly at the surface of the water.

A quantity of air is also taken in under the hemelytra which, with the concave dorsum of the abdomen, forms an efficient chamber for the storage of air. This air the insect respires when at the bottom of the water. After a time the subelytral air becomes gradually impure and is removed. The insect moves the retractile organs rapidly in and out, bringing out every time a minute bubble of air from under the hemelytra with the help of the backwardly projecting hairs on the organs. The bubbles collect at the tip of the abdomen and when large enough escapes out of the water. Two or three such bubbles are given off one after another sometimes before the insect goes to the surface for a renewal of the storage air. For the ejection of one bubble, it may take as much as a full minute. At times a bubble is not easily released and the insect then brushes it off with its hind legs.

The retractile organs therefore serve two main functions. It enables the insect : (1) to breathe direct atmospheric air through the eighth abdominal spiracle ; and (2) to force out mechanically bubbles of impure air from the subelytral chamber.

In the Nepidæ the eighth abdominal segment has been modified to form a long siphon-like tube made up of two halves. This siphon is long and is not capable of being retracted as such. The two valves of the siphon, how-ever, may be seen occasionally to move alternately slightly backwards and forwards like the action of a piston. The inner side of the siphon is covered

with backwardly projecting hairs and the moving of the two valves alternately helps to bring out small bubbles of air from the subelytral chamber to the tip of the siphon where they collect into a big bubble which is eventually released. This was observed in *Laccotrephes* and *Ranatra*. Curiously enough previous workers on Nepidæ have not observed this fact. The siphon of the Nepidæ therefore is both homologous and analogous to the retractile organs of the Belostomatidæ. Schiödte believed the retractile organs of the Belostomatidæ to have some genital function having nothing to do with respiration.

5. *The External Air Storage.*

Reference has already been made to the air carrying habit of the insect for the purpose of breathing when entirely submerged. Air is stored on the abdomen under the hemelytra as well as under the sides of the pronotum. The dorsum of the abdomen is very much concave and is covered over by the slightly convex hemelytra. The hemelytra are locked firmly to the body along the sides of the metathorax and the scutellum. Both the hemelytra are again perfectly interlocked and are never opened by the insect. Thus an efficient chamber is formed for the storage of air. In the degenerate winged form the reduction of the inner wings makes the chamber slightly more spacious. The first abdominal spiracles are dorsal and open directly into this chamber. To the metathoracic and the supernumerary spiracles air is led from this chamber by side channels formed by the upcurving of the mesopleura (Figs. 2 and 3).

The mesothoracic spiracles placed ventrally on the prothorax open into large concavities. These are in communication with the mesopleural channels by small openings. But air can also be taken in at the surface of the water below the posterior margin of the pronotum by the bending forwards of the prothorax. The posterior margin of the pronotum is fringed with short hairs which prevent water from getting in.

6. *The Mode of Respiration.*

The function of the subelytral air and breathing when submerged in water.—The function of the subelytral air of aquatic insects has been a matter of some difference of opinion among workers. Brocher, for instance, was of opinion that the storage air was merely hydrostatic and that it was the air passed out by the insect through the thoracic spiracles and collected under the hemelytra, and not the air taken in from the atmosphere. He believed that the air was meant only to enable the insect to reach the surface easily. However, he conceded that when necessary this air may be taken in again in respiration. Wigglesworth in his recent review on the respiration of insects discusses at some length the part played by the air stores of aquatic insects. Two main functions have been attributed to this air, *viz.*,

hydrostatic and respiratory, and Wigglesworth believes them both to have been sufficiently proved.

Our observations on *Sphærodema* show that when at the bottom of the water the insect gives out one or two large bubbles of air from under the hemelytra by rapidly moving the retractile organs. The insect then goes up to the surface for a gulp of the atmospheric air and rushes down immediately to return to the bottom. The removal of air from the subelytral space prior to going up to the surface is a handicap for going up and it would not have been done if this air had any hydrostatic function. The specific gravity of *Sphærodema* taken both with and without the storage air is slightly less than that of water and so the insect can easily rise up to the surface of the water. That is what is observed ; for even when the hemelytra are removed and with them the storage air the insect floats without any effort. On the contrary when going down to the bottom the insect has to swim down actively and can remain at the bottom only by clinging to some object with its legs. Thus little recognition can be given to the hydrostatic function attributed to the subelytral air.

Observations on *Sphærodema* show that the subelytral chamber is filled with fresh air at the surface of the water directly from the atmosphere. When the insect comes to the surface of the water it raises slightly the tips of its hemelytra with the help of the retractile organs and so brings the subelytral chamber into communication with the atmosphere. As some air from this chamber has been previously removed below water, the atmospheric air gets in to take its place. The insect may continue to stay at the surface or may immediately go back to the bottom. As only the spiracles on the thorax are in communication with the subelytral air as already described, it is natural to assume that these spiracles are used for breathing. Experiments have testified to the correctness of this view. When the insects were prevented by removing the hemelytra from storing air under them they died in a very short time. When only the distal halves of the hemelytra were cut off, or only a single hemelytron was removed, the insect remained alive though seemingly somewhat uncomfortable. It then could not easily remove the storage air when it became impure and had to come to the surface often to do so, or struggle with its hind legs in trying to brush off the impure air. When the hemelytra were entirely removed, if floating supports were placed in the water the insect readily made use of them to raise itself up right out of water in order to bring the thoracic spiracles into direct communication with the atmosphere and continued to live in this way for a few days at least. These observations prove that the insect cannot do without breathing through the thoracic spiracles.

A third function has been attributed to the storage air. According to Wigglesworth, Comstock (1887) from observations on Corixa, first suggested that the film of air carried by the insect might serve as a gill, diffusing oxygen from the water. Dogs (1908) seems to have expressed a similar view for *Nepa*. This view may be applicable in the case of the nymph where the film of air is carried on the ventral side of the abdomen in touch with the surrounding water medium. But in the adult the storage air is enclosed by the hemelytra and is unexposed to the water. The possibility of diffusion of gases under the circumstances would be unthinkable.

It has been seen when dealing with the spiracles that the metathoracic spiracle and the supernumerary spiracle open into two distinct compartments of the mesopleural air channel, and that the supernumerary spiracle is an exhaling spiracle as suggested by its structure and action. It is assumed therefrom that the impure air given out by the supernumerary spiracle travels backwards through the outer channel and the air from the subelytral space travels forwards to the metathoracic spiracle through the inner channel. We have no direct evidence to show that the other spiracles on the thorax are only inspiratory in function. But there are three factors which have to be critically examined : (1) If the same spiracle were to inspire and expire air, there would be impure air in the immediate vicinity of the spiracle which is not a desirable thing. (2) The supernumerary spiracle which is an expiratory one is isolated (physiologically) from the others. (3) The body wall of the abdomen is capable of expiration and that in order to have an adequate balance between expiration and inspiration there must be some spiracles solely set apart to perform the latter function. We are therefore forced to assume that the mesothoracic, metathoracic and first abdominal spiracles are purely inspiratory in function.

Expiration through the dorsal abdominal wall.—Sphærodema has been observed to give out small bubbles of air through its abdominal terga. The abdominal terga are very thin-walled and transparent and in some spots, particularly less chitinized. If the insect is placed on its back in a small dish of water after cutting off the hemelytra, bubbles of air will gather below in the abdominal region. The gradual formation of these bubbles could be clearly observed through a binocular microscope. Brocher observed similar bubbles of air adhering to the abdomen of *Nepa*. He believed them to have been given out by the water itself. He was not however happy about this explanation for he mentions that in nature the insect lives in situations where there would be no chance of the water being well aerated. We have used water from which dissolved air was driven out by boiling and still could see the bubbles appearing on the insect. Further, a dead specimen

does not show any such phenomenon. We believe this is the first time such an observation has been recorded.

It has already been mentioned in dealing with the spiracles that from the bulbous trachea of the mesothorax, part of the air escapes into the body cavity. This is true of *Laccotrephes* as well. This air, we presume, diffuses out of the body wall. Air is possibly passed into the blood by other organs and finds an exit through the body wall. If this be so the " false spiracle " of *Laccotrephes* is only a more specialised spot on the terga for giving out air. The subject will be fully discussed later.

Breathing on the surface of the water.—When on the surface *Sphærodema* rests in an oblique, head downward position. It then pierces the surface film with its retractile organs and breathes directly from the atmosphere by means of the last pair of abdominal spiracles. The retractile organs are covered with long hydrofuge hairs to which air' particles adhere and are directed to the spiracles which remain hidden. By themselves these spiracles are too inadequate to meet the needs of the animal and without the thoracic spiracles functioning also, as shown above, the insect cannot live. Brocher believed that the last pair of abdominal spiracles alone functioned for taking in air and that the thoracic spiracles functioned only to give out air. It is however unreasonable to think that only one small pair of spiracles would be used as inhaling organs while three pairs of large spiracles were used as exhaling organs. Even when *Sphærodema* is on the surface breathing
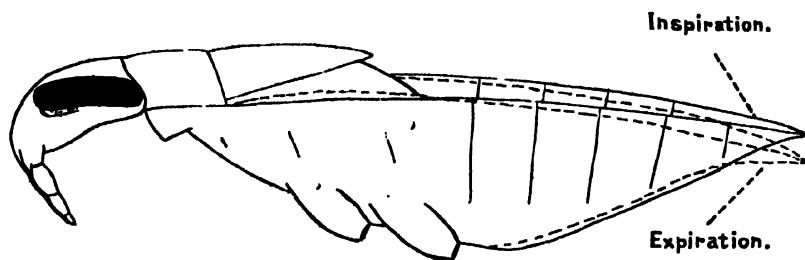

FIG. 11. Adult showing the respiratory movement of the abdomen.

directly from the atmosphere it makes use of the thoracic spiracles as the subelytral chamber remains filled with air and this again is in communication with the atmosphere. The general habit of the insect shows that it remains mostly at the bottom of the water and comes up to the surface only at intervals for a renewal of the storage air. It would be impossible for the last pair of abdominal spiracles to take in all the air required in one gulp. The occasional coming to the surface, therefore, is undoubtedly to renew the subelytral air only and this is always preceded by giving out bubbles of air

under water as already stated. When under water the last pair of abdominal spiracles would function only to give out air.

The movement of air in the abdominal tracheal channels during respiration.—The direction the air takes in the abdominal spiracular branches (S.B., Figs. 1 and 12) during respiration under water is well observed on account of the more or less transparent wall of the abdomen. The respiratory movement involves a ventral deflection of the posterior half of the abdomen which results in a dorso-ventral contraction of the abdomen (Fig. 11). At this stroke, air in the abdominal trunk (A.T.) is forced into the spiracular branches (S.B.). When the abdomen relaxes, a part of the air is passed into the smaller branches of the spiracular tracheæ and a part gets back to the main trunk. (Fig. 12 illustrates the course of air in a typical abdominal segment. The dark arrows show the course of air at the deflection
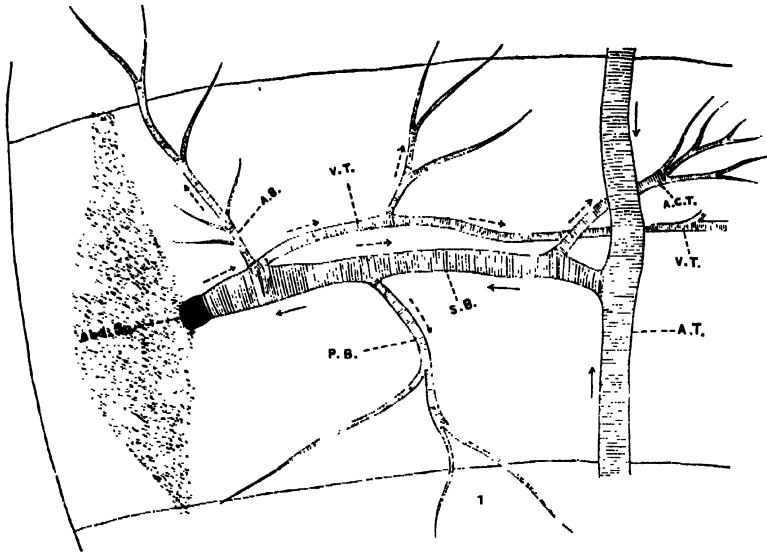

FIG. 12. The tracheation of a typical abdominal segment and the course of the circulation of air in that segment. The dark arrows show the course of air at the expiratory movement of the abdomen. The dotted arrows show the course of air at the inspiratory movement of the abdomen.

of the abdomen ; the dotted arrows, at the relaxation of the abdomen.) As the spiracles in the abdomen are closed the deflection of the abdomen, which is an expiratory act, does not produce the desired effect in that region of the body except through the last abdominal spiracle.

In the thorax the only spiracle that could be seen giving out air is the supernumerary spiracle. The hood-like membrane of that expands and it

is assumed that air escapes through it. There is no indication whatsoever that others also give out air. On the other hand we have given elsewhere reasons for believing that they are only inspiratory in function.

The rectal cæcum and respiration.—The rhythmic peristalsis of the rectal cæcum, a structure present only in aquatic insects, has led to different interpretations as to its function. Such a contraction naturally suggests a respiratory or circulatory function. The histological structure of the cæcum does not indicate that it might function as a tracheal gill. It was suspected that it might take in and give out water but careful observations have not revealed the presence of a current of water through the anus. We have given in another paper reasons for not accepting the views of previous workers. To us it appears that the cæcum helps in the ventilation of the tracheal system. The cæcum is a distended tube, has a median position in the body cavity and extends upto the mesothorax. As it contracts the air in the longitudinal tracheæ seems to move.

III. The Respiratory System of the Nymph.

1. The Tracheal System and Spiracles.

The general arrangement of the tracheæ in the nymph is similar to that of the adult. There are ten pairs of spiracles which are all ventrally situated and all are functional (Fig. 13). The thoracic spiracles are similar in structure to those of the adult but their situations vary. The first pair, the mesothoracic spiracles, is situated on the wall of the main thoracic tracheal trunk as in the adult. The second pair, the metathoracic spiracles, is ventral and is protected by the epimera of the meso-sternum. The supernumerary spiracle of the adult and the bulbous trachea associated with it are absent. The third pair of spiracles, the first abdominal, is also ventral and is protected by outgrowths from the epimera of the meta-sternum. The second abdominal spiracle opens on the first abdominal sternum and is similar in structure to the thoracic spiracles. The abdominal spiracles three to eight open on the sterna of their respective segments. In structure they are similar to the eighth abdominal spiracle of the adult (Fig. 9). The eighth abdominal segment is visible in the nymph and there are no retractile organs. The air sacs and the " tracheo-parenchymatous organs " of the full winged form are absent in the nymph.

2. The Air Flaps (Fig. 13).

In the nymph all the spiracles are ventral as mentioned above. The problem of respiration under water is therefore solved in a way different to what obtains in the adult. The ventral side of the metathorax has developed thin, transparent, semi-circular outgrowths from the epimera of the
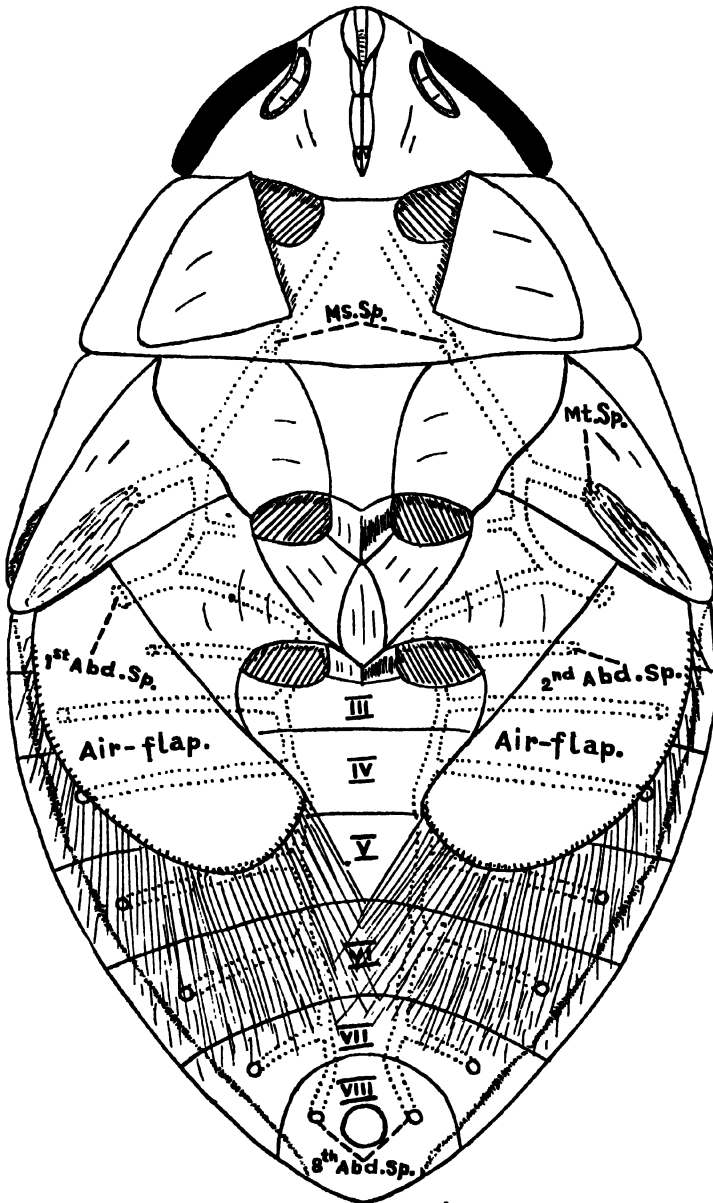
FIG. 13.   Ventral view of the nymph showing the air-flaps, the spiracles, and the main tracheæ.

meta-sternum.   These are here referred to as "air-flaps".   They extend over a great part of the abdomen reaching to the middle of the fifth abdominal segment.   Their semi-circular distal margins bear a row of short spines

and a row of very long fine hairs. These hairs form a close matting which extend over the posterior half of the abdomen. The cavity, thus enclosed by the air-flaps and their hairs, serves as the air chamber of the nymph. Anteriorly this cavity is continued to the mesothoracic region where the flap-like epimera of the meso-sternum encloses it. The spiracles open into this cavity and are thus in direct communication with the storage air. The air-flaps are supplied with a few very fine tracheoles.

Martin (1896) and Bueno (1906) recorded these flaps in the nymphs of Belostomatidæ. According to Martin, Dufour in his monograph on the Belostomatidæ, had also described these flaps in the nymph of *Hydrocyrius algeriensis* : " Ventris squama basilaris magna duplex semicircularis." In the Nepidæ the metasternal flaps are not developed, but the epimera of the mesosternum are much more elongated and run as narrow strips along the margins of the abdomen.

About the function of these flaps Martin was of opinion that they re-called the caudal gills of the larvæ of Agrion and likewise served as tracheal gills. According to Bueno "they may be used for the storage of air, or per-haps as a means of reducing the quantity held by the pile, by exercising pressure to force it out". Our conclusions are, that these flaps serve the same purpose as do the hemelytra of the adult, *viz.*, to store air for respira-tion. When these flaps were entirely removed the nymphs of *Sphærodema* very soon died as the spiracles no longer functioned being blocked up by the water. But, as in the case of the adults, when floating supports were pro-vided the nymphs made use of them to raise themselves well out of water keeping their spiracles in contact with the atmosphere and thus remaining alive. When only half of the air-flaps were cut off the nymphs were able to store air in the remaining half and continued to live, as did the adults with half the hemelytra cut off. This clearly shows that the flaps are merely for holding air for breathing by means of the spiracles that open into them.

3. *The Mode of Respiration.*

From the foregoing it is clear that the respiratory mechanism is different from that of the adult. When the nymphs are observed swimming about in the water a glistening film of air is always noticed covering the under-surface of the abdomen. The ventral side of the abdomen is covered with long hydrofuge hairs and a quantity of air could be held between them. Air could also be stored under the metasternal air-flaps and the mesosternal epimera. The long projecting hairs of the air-flaps and the hydrofuge hairs on the abdomen working in combination hold the air bubble referred to above. The purpose of this air bubble is the same as that of the storage air under the hemelytra of the adult. The air-flaps are therefore analogous in this

respect to the hemelytra. Not only is the air held by the nymph necessary for its respiration but it is also useful as a means of protecting the ventral spiracles from being blocked up with water. The nymph takes in air by all the ten pairs of spiracles whether submerged in water or at the surface.

As in the adult, air particles are observed to diffuse out of the body wall of the abdomen. But here it is the sterna, which are thin walled and transparent, that exhibit this phenomenon. The phenomenon is better observed in this case than in the adult, the nymph being more transparent than the adult. It is clearly noticeable when the ventral surface of the abdomen is in contact with water in a small dish placed under the microscope. When the storage air under the abdomen becomes gradually impure the nymph brushes off the film with its hind legs and ascends at once to the surface for fresh air. The tip of the abdomen is raised to the surface film and the hydrofuge chamber comes in contact with the atmospheric air. Thus a fresh store is taken in. The retractile organs are not developed in the nymph and are not needed.

The suggestion, that the rhythmic movements of the rectal cæcum in the adult help to ventilate the larger tracheæ, also applies to the nymph as its cæcum behaves in a like manner.

IV. General Considerations.

A large number of adult insects that have secondarily taken to an aquatic life, have exploited the subelytral space for storage of air which they breathe during submersion. It cannot be claimed that the bugs alone have developed this. The beetles have independently developed almost the same condition.

1. *Adaptations to Aquatic Life.*   .

The perfection of adaptation to aquatic life depends on : (1) rendering the subelytral chamber an effective air. store for breathing purposes under water ; and (2) developing a mechanism for the removal of the impure air from the body as well as from the air storage.

The subelytral chamber.—The subelytral space in *Belostoma* is not a permanently closed chamber as in *Sphærodema,* since both the pairs of wings are used in flight. The subelytral chamber of the Nepidæ (*Laccotrephes, Ranatra,* etc.) is closed but is not so spacious as in *Sphærodema,* since the dorsum of the Nepidæ is only slightly concave though the margins are more deeply grooved. *Sphærodema* therefore is able to store a larger quantity of air than *Laccotrephes* or *Ranatra.* The degenerate winged form of *Sphærodema rusticum* possesses a chamber slightly more spacious, due to the reduction of the inner wings, than the normal winged form, in which the

large folded inner wings occupy some space. In *Sphærodema* the hemelytra are interlocked and are closely apposed to the abdomen. In the Nepidæ the hemelytra are locked to the abdomen there being no posterior interlocking arrangement between the pair.

The removal of impure air by the retractile organs.—The retractile organs are used for the removal of air from the subelytral air store. *Nepa, Ranatra*, etc., have gone a step further in the evolution of these organs. The strap-shaped, short retractile organs of *Sphærodema, Belostoma*, etc., have become a siphon-like tube in the Nepidæ. The siphon is made up of two halves, the concave inner surfaces of which are clothed with fine hairs directed backwards. At the base of the siphon lies the last pair of abdominal spiracles, the terminations of the main longitudinal tracheæ. Like the retractile organs, besides helping to take air at the surface, the siphon also removes the air from under the hemelytra at intervals. The two halves of the siphon rub against each other, each being drawn in a little and pushed out alternately. The hairy inner surface of the siphon brushes out small bubbles of air to the tip where they are collected into a large bubble and subsequently released. We have already referred to the fact that this function of the siphon has not been recorded by previous workers who attributed to it, only that of exposing the last pair of abdominal spiracles to the surface of the water.

The removal of impure air by the body wall.—Though respiration through the integument has been known to exist in insects [*vide* Muttkowski (1920), Wigglesworth and others] the profuse diffusion of air through the abdominal body wall, as it occurs in *Sphærodema*, and we believe also in *Nepa* and other water-bugs, has not been noted by previous workers. Muttkowski showed that the insect chitin is permeable to gases, especially to CO_2. Brocher noted bubbles of air on the abdomen of *Nepa* but he believed them to be air which escaped from the surrounding water medium adhering to the hairy surface of the abdomen. We have shown from observations and experiments on *Sphærodema*, that these bubbles diffuse out of the body of the insect.

2. *Loss of Flight.*

The aquatic Hemiptera exhibit many interesting intermediate stages in the degeneration of their power of flight as a consequence of the adaptation of the hemelytra for the storage of air. *Belostoma* is an actively flying insect and can take to flight whenever it is necessary. *Sphærodema rusticum* on the other hand, though belonging to the same family Belostomatidæ, has lost its power of flight. The indirect wing muscles have either been completely suppressed or have become degenerate. The right and the left hemelytra are almost inseparably locked, a feature which restricts flight.

In the case of the degenerate winged form the evolution is more complete. The elytra have become almost completely coreacious and the inner wings have lost their anal regions. It is not known if all the species of *Sphærodema* show the same degree of degeneration in structure as *S. rusticum*, but as far as *S. rusticum* is concerned, it can be safely said that it is a creature more adapted than *Belostoma* for aquatic life. According to Bueno (1916), *Plea* has become more specialized in this respect having lost the second pair of wings completely and the first pair having become entirely coreacious and soldered together. Species of *Plea* collected here showed fully developed inner wings and the above remark may have to be accepted with reservation.

The Nepidæ have also lost their power of flight as their indirect wing muscles have degenerated into the " tracheo-parenchymatous " organs. But Ferrière and Poisson have recorded specimens of *Nepa* with normal indirect wing muscles. It is quite possible therefore that, as suspected in the case of *Sphærodema*, occasionally a few individuals may show the power of flight.

3. *The Rôle of Blood in Respiration.*

A very novel problem, till now unsuspected, presents itself from the fact that aquatic insects, as shown in *Sphærodema*, are capable of diffusing out air through the chitinous membrane of the body wall. The tissue that is in immediate touch with the body wall is the blood and it is reasonable to assume that most of the air diffuses out from that tissue. How exactly it is capable of liberating this air is not known and has to be investigated. This observation however recalls to mind the observations of Tillyard and Frankenberg on the first filling of the tracheæ with air in the submerged newly hatched nymphs of the dragon fly and *Corethra*. They have shown that the air which fills the tracheæ comes from the blood. Coupled with this are the findings of Muttkowski on the rôle of insect blood in respiration. From the evidence thus available there can be no great objection to assuming that the air which escapes through the body wall in *Sphærodema* comes from the blood. Air from the tracheæ escapes to the blood and from the blood to the outside through the body wall. It has on its way to supply oxygen to that tissue and the fat bodies and to absorb CO_2. The passage of air through the blood is evidently a contrivance to oxygenate the blood and also to deprive it of CO_2. It is presumed therefore that the blood of aquatic insects probably has a greater respiratory function than the blood of other insects.

4. *The Probable Line of Evolution of the Aquatic Adaptations.*

From a comparative study of the adaptations for breathing air in the adult and the nymph of *Sphærodema* a question arises as to why different

arrangements were necessary and further as to which of the two was the earlier. Similar differences are also present in the Nepidæ and Maulik in his paper on *Nepa* suggests an answer to this question. He says that the adult *Nepa* having to perform the function of reproduction is compelled to remain under water for a considerably long time and so has developed a long siphon to enable it to take in air from a distance away from the surface of the water. We have shown elsewhere that the retractile organs of *Sphærodema* are homologous and analogous to the respiratory siphon of *Nepa*, and that they are developed primarily not for taking in air but to aid the insect in getting rid of the air under the hemelytra when it becomes impure. In the circumstances Maulik's suggestion appears untenable.

Speculations as to the origin of aquatic life in this group of insects may yield valuable results. In the remote past as competition on land became severe, the carnivorous bugs would have been compelled to explore new avenues of food supply and fresh water might have afforded a good field. At first, visits might have been strictly limited to the border and as perambulation in water was needed, the adults would have been able to breathe for a little while under water with the single pair of dorsal spiracles on the metathorax, as some air could be conserved between the wings and the body. In the earlier stages this one pair of spiracles would have been sufficient for the purpose. (The dorsal arrangement of the spiracles on the metathorax is not an acquirement for the aquatic habit ; Schiödte describes it as lying on the back of the insect, hidden by the wings between the metanotum and the first dorsal segment of the abdomen in all Heteroptera.) Later on as the period of submersion had to be extended, the subelytral space was thoroughly exploited to store up air, and this is found in different degrees in *Belostoma* and *Sphærodema*. To overcome the difficulty of going back to land every time the insect wanted to remove the impure air by opening its wings, the adult might have developed the retractile organs which serve to remove the storage air. These structures also helped the insect to take in fresh air at the surface of the water, the last pair of abdominal spiracles being placed at their base. Thus the retractile organs of the Belostomatidæ came to be evolved. The siphon of the Nepidæ, we have shown, is a more efficient modification of these retractile organs. Finding water a more profitable source of food these bugs might have strengthened their aquatic habits. The spiracle on the mesothorax was pushed to the dorsal side by the curving up of the mesopleura and brought into communication with the air under the hemelytra. The spiracle on the prothorax though ventrally situated was made functional with access to air from the dorsal side. Thus the three pairs of spiracles on the thorax and one on the base of the retractile organs served the insect for its

respiration. All the other spiracles on the abdomen which were ventral became non-functional and consequently were blocked.

To take in and give out air from the same spiracles in a closed chamber though quite useful in expediency was not a great success. It meant that most of the same air that was given out by the spiracles was immediately taken back. Means for remedying this defect were devised, and the result is the more perfect respiratory mechanism found in *Sphærodema*, the details of which need not be repeated here.

For the nymph it might have been a more difficult problem. When the adult paid visits to the water it naturally laid eggs on floating plants or on those on the banks and the nymph therefore had to adapt itself to the new surroundings. Being wingless it had no means of storing air and all its spiracles were ventrally placed. It had therefore to carry air on its ventral side. It may be imagined that at first for short trips to the water the ancestral nymph would have bent its legs against the abdomen in such a way as to prevent the spiracles from being wetted by holding a little film of air. This might have prevented the insect from using its legs freely in swimming ; so gradually the sternal flaps with the long hairs were developed. The hind legs could easily brush off the bubble of air from the ventral side of the abdomen and other structures were not necessary. All the spiracles therefore remained open and functional, being prevented from coming into contact with water by the film of air held by the air-flaps.

To follow the story of the gradual adaptation to aquatic life, continued existence in water coupled with the exploitation of the subelytral air space as a storage chamber necessitated the gradual suppression of flight in most of these insects. The hemelytra became more and more adapted to the function of forming a water-tight chamber for the storage of air and became firmly locked to the abdomen and to each other. The muscles of flight lost their function as the fibres began to degenerate. The degenerate winged form of *Sphærodema* indicates perhaps a probable line of future evolution in this insect.

V. *Summary and Conclusion.*

1. Two forms of adult *Sphærodema rusticum* with peculiar alary dimorphism are recorded ; one with normal wings and the other with degenerate wings (the membrane region of the hemelytra and the anal region of the inner wings being absent). Both have lost their power of flight.

2. The respiratory system of the two forms differs in certain respects. The normal winged form possesses "tracheo-parenchymatous organs" and air sacs in the thorax, which are wanting in the degenerate winged form.

3. The " tracheo-parenchymatous organs " are modified indirect wing muscles. The histology of the organs does not show the transverse striations of the normal muscle fibres. The phagocytes noted by Poisson in these organs in other aquatic Hemiptera are not recognised in this insect. The tracheal supply of these organs is not the normal tracheal supply of the wing muscles as Ferrière believed for *Nepa*, etc., for in *Sphærodema* there are more and larger tracheæ in these organs. Experiments conducted prove that the degenerate winged form is not any the worse for want of these structures.

4. The respiratory system of the adult and the nymph of *Sphærodema* differs in some respects though the general arrangement of the tracheæ is similar in both. These variations are traceable to the individual respiratory needs for aquatic habits.

5. All spiracles of the nymph are ventrally situated and all are functional. In the adult the metathoracic and the first abdominal spiracles have become dorsal and the others remain ventral. Only four pairs of spiracles are functional in the adult, the three on the thorax and the last abdominal. The last abdominal spiracles are borne by the retractile organs in the adult.

6. The first pair of (mesothoracic) spiracles of both the adult and the nymph are peculiar in being on the wall of a trachea and not at the termination as is usual. They are so situated in *Nepa*, *Ranatra*, etc., but the fact has not been made known generally.

7. The three pairs of abdominal spiracles which are modified into the so-called sense organs in the Nepidæ are not so modified in *Sphærodema* and *Belostoma*.

8. The adult *Sphærodema* possesses in addition to the second pair of (metathoracic) spiracles, a supernumerary pair on the same segment. The hood-like membranous covering of this spiracle is observed to expand at every exhalation. It is shown that this supernumerary spiracle functions only as an exhaling organ and the true metathoracic spiracle, from which it is separated by a partition, functions only as an inhaling organ. This supernumerary spiracle is also present in *Belostoma, Nepa, Laccotrephes, Ranatra*, etc., but its nature and function have not been correctly noted and interpreted before.

In the Nepidæ in addition to this supernumerary pair of spiracles on the mesothorax, there is a pair of somewhat similar structures (the " false spiracles " of Hamilton) on the metathorax. Although this second pair is without tracheal connection, it is pointed out that it behaves in a similar manner to the above-mentioned supernumerary spiracles.

9. Air from the body is given out through the dorsal abdominal body wall of the adult and the ventral abdominal body wall of the nymph. Bubbles of air have been observed to diffuse out of these regions. Brocher observed such bubbles on the abdomen of *Nepa* but he thought them to be air bubbles liberated by the surrounding water medium and caught in the pile on the insect abdomen. We have shown that it is not so.

10. That insect blood plays a part in respiration has been recently recognised. From the profuse diffusion of air from the body wall we presume that the blood of aquatic insects has a greater respiratory activity than that of terrestrial insects.

11. The function of the subelytral air which is carried by the adult water-bugs is shown from the study of *Sphærodema* to be purely respiratory contrary to the findings of Brocher and others. The locking arrangements of the hemelytra help to make a water-tight chamber on the dorsal side of the abdomen for storing air. The air storage organs of the nymph are a pair of large semi-circular outgrowths of the meta-epimera, with long projecting hairs which cover the major portion of the abdomen. The air held by these flaps serves the same function as the storage air of the adult. All the ten pairs of nymphal spiracles use this air in breathing.

12. For removing the subelytral air the adult has developed two strap-shaped retractile organs from its eighth abdominal segment. As the spiracles of the eighth segment open on them, the organs when thrust out bring them into communication with the atmosphere at the surface of the water and hence make them functional. The retractile organs of the Belostomatidæ are both homologous and analogous to the respiratory siphon of the Nepidæ. The air-removing function of this siphon has not been noted by others but observations on *Laccotrephes* and *Ranatra* have made it possible for us to analogise this siphon with the retractile organs. The nymph brushes off bubbles from the storage air which is held on its ventral side with its hind legs.

13. The course of the air in the abdominal tracheæ during respiration is described.

14. To the rhythmic contractions of the rectal cæcum is attributed a tracheal ventilatory function.

15. The changes from the nymph to the adult adaptations are sudden and take place in the last moult. They include the loss of the air-flaps of the nymph, the shifting of the metathoracic and the first abdominal spiracles to the dorsal side, the development of the supernumerary spiracle and the retractile organs, and the blocking up of the ventral spiracles.

16. The probable line of evolution of the aquatic adaptations in the different aquatic bugs is discussed.

Acknowledgments.

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A.B.   ..   Anterior Branch of the abdominal spiracular trachea.

Abd.Sp.   ..   Abdominal Spiracle.

A.C.T.   ..   Alimentary Canal Trachea.

A.T.   ..   Abdominal Trunk trachea.

B.T.   ..   Bulbous Trachea of the mesothorax.

C.R.   ..   Chitinous Ridge dividing the mesopleural air channel.

D.B.   ..   Dorsal Branch of the thoracic spiracular trunk trachea.

D.T.O.   ..   Dorso-longitudinal Tracheo-parenchymatous Organs.

H.T.   ..   Head Trachea.

I.C.   ..   Inner Compartment of the mesopleural air channel.

K.   ..   Club-shaped trachea of the mesothorax.

L.T.   ..   Longitudinal Trachea of the thorax.

Ms.D.T.   ..   Mesothoracic Diagonal Trachea.

Photomicrograph of T. S. of tracheo-parenchymatous organ. ×60

Photomicrograph of T. S. of tracheo-parenchymatous organ. ×150

Photomicrograph of the hind portion of the abdomen
of adult showing the retractile organs.

Ms.L.T.	..	Mesothoracic Leg Trachea.
Ms.Pl.	..	Mesopleuron.
Ms.Sp.	..	Mesothoracic Spiracle.
Ms.T.C.	..	Mesothoracic Tracheal Cross.
Mt.L.T.	..	Metathoracic Leg Trachea.
Mt.Sp.	..	Metathoracic Spiracle.
O.C.	..	Outer Compartment of the mesopleural air channel.
P.B.	..	Posterior Branch of the abdominal spiracular trachea.
Pr.D.T.	..	Prothoracic Diagonal Trachea.
Pr.L.T.1.	..	Prothoracic Leg Trachea, one.
Pr.L.T.2.	..	Prothoracic Leg Trachea, two.
Pr.T.C.	..	Prothoracic Tracheal Cross.
R.O.	..	Retractile Organs.
R.T.	..	Rostral Trachea.
S.B.	..	Spiracular Branch trachea of the abdomen.
S.G.T.	..	Salivary Gland Trachea.
S.Sp.	..	Supernumerary Spiracle.
S.T.	..	Spiracular Trunk trachea of the thorax.
S.T.O.	..	Subsidiary Tracheo-parenchymatous Organs.
T.B.	..	Transverse Branch trachea of the thorax.
T.T.	..	Thoracic Trunk Trachea.
V.B.	..	Ventral Branch of the thoracic spiracular trunk.
V.C.	..	Ventral Commissural trachea of the 7th Abdominal Segment.
V.T.	..	Ventral Trachea of the abdomen.

A DILATOMETRIC METHOD FOR STUDYING THE " IN VITRO " DIGESTIBILITY OF MILKS.

BY (MISS) KAMALA BHAGVAT
AND
MOTNAHALLI SREENIVASAYA.
(*From the Department of Biochemistry, Indian Institute of Science, Bangalore.*)

IN the course of a comparative study of the digestibility of milks subjected to various physical and chemical treatments and from different species of animals, it was found necessary to develop an easily reproducible and accurate method of following the course of digestion. In a previous communication (1935) it was shown that the relative digestibility of proteins "in vitro" can be determined by the dilatometric method (1932) which possesses the additional advantage of effecting considerable economy of research material, a circumstance of great importance in the case of those milks which are available only in limited quantities.

There is an obvious difference between the casein particle in milk and that in an artificially prepared solution of casein. This difference in size, state of aggregation and hydration, is expected to influence the rate of enzymic digestion. In milk, the solution of lactose, salts and the non-protein nitrogen molecularly dispersed in water, offers the stabilising medium for the colloidally dispersed casein particle ; in the case of the artificial casein solution, the protein is peptised in a suitable buffer. The present communication relates to a study of the casein particle in its natural environment as compared with the digestion of an artificial solution of casein, by two independent methods, chemical and physical.

Experimental.

Cow's milk centrifuged for 30 minutes at 3000 r.p.m. for skimming off fat, was employed after buffering with half molar concentrations of Sorensen's phosphates to yield a p_H of 7·7. By employing this high concentration of buffer, the extent of dilution of milk was kept low. 260 ml. of milk made up to 300 ml. with the buffer, represented the "full concentration" stock solution. Other concentrations of milk, one-half, one-third and one-sixth, were prepared by diluting the stock solution with M/15 phosphate buffer of p_H 7·7.

The casein solution was prepared by dissolving Hammerstein's casein, previously ground and wetted with water, in phosphate buffer of p_H 7·7.

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Slight warming to 40° C., facilitated solution. A six per cent solution of casein, thus prepared, served as the stock solution, which was accurately standardised by a determination of the total nitrogen by Kjeldahl. Solutions of casein, corresponding respectively to the nitrogen content of the different concentrations of milk, were prepared by diluting calculated amounts of the stock solution with M/15 phosphate buffer of p_u 7·7.

A two per cent. solution of Pfansteihl's trypsin was prepared in 7·7 p_u phosphate buffer. The reaction mixture for the dilatometer consisted of 50 ml. of the substrate and 5 ml. of the enzyme and the reaction was independently carried out in a separate flask, from which aliquots, at definite
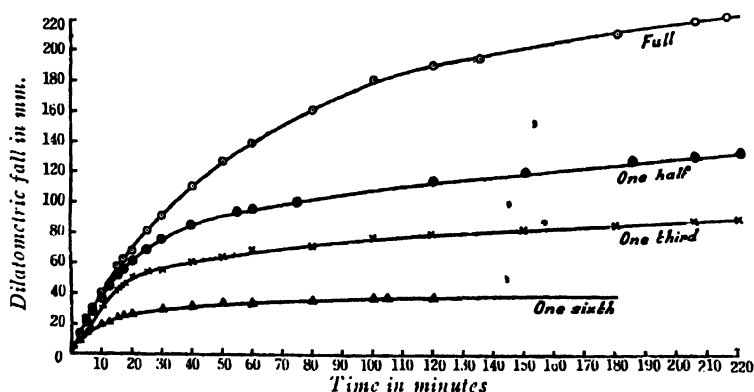

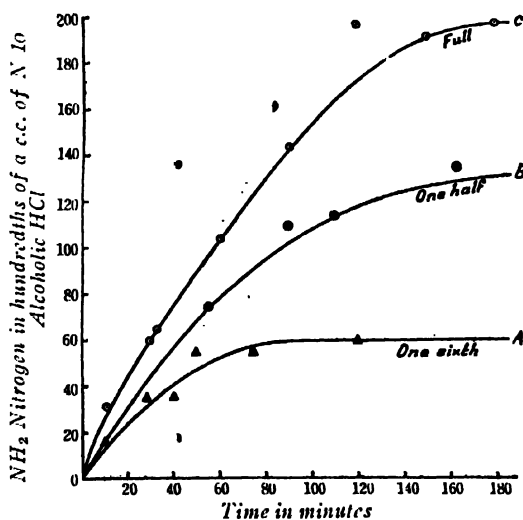FIG. 1.   Digestion of Cow's Milk.
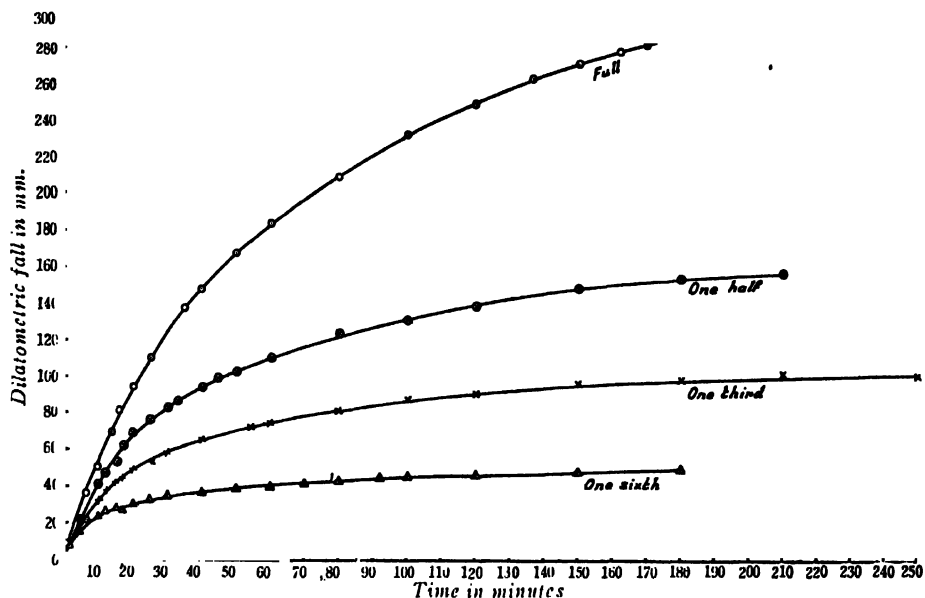

FIG. 2.   Digestion of Cow's Milk.
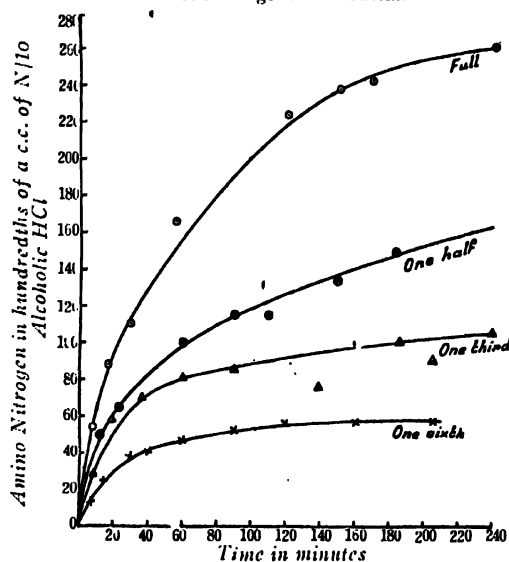
FIG. 3.   Digestion of Casein.

FIG. 4.   Digestion of Casein.

intervals, were drawn and the amino nitrogen estimated by Linderstrom Lang's acetone titration. The dilatometric depressions are measured in mm. representing the linear fall of the capillary column and the increases in amino nitrogen expressed in hundredths of a ml. of decinormal alcoholic HCl.

Discussion.

The experimental values are graphically represented in Figs. 1, 2, 3 and 4. The dilatometric depressions at definite intervals of time are taken from the graph and expressed in μl while the corresponding values for amino nitrogen taken from the graph, are expressed in mgms. of amino nitrogen. Tables I and II incorporate these values while Figs. 5 and 6 represent the correlation between the two sets of values, the dilatometric depression and amino nitrogen for milk and casein, each for three different concentrations.

TABLE I.

Milk.

Substrate Concentration		Time in Minutes									
		10	20	30	60	90	120	150	180	210	240
One-sixth	Mgms.	2·00	3·90	5·30	7·80	8·80	8·80	..	..	..	..
	μl	2·37	3·21	3·56	3·92	4·27	4·63	..	..	..	..
One-half	Mgms.	2·7	5·0	7·0	12·0	16·0	18·2	19·2	19·8	19·8	..
	μl	4·39	7·0	8·78	11·4	12·47	13·53	14·24	14·95	15·55	18·67
Full	Mgms.	3·80	6·70	9·10	16·10	21·20	25·20	28·40	29·20	29·80	29·80
	μl	4·75	8·07	10·80	16·5	20·53	22·68	23·86	25·17	25·80	26·36

TABLE II.

Casein.

Substance Concentration		Time in Minutes						
		10	20	30	60	90	120	150	180
One-sixth	Mgms.	3·07	4·67	5·26	5·55	7·00	7·74	8·18	..
	μl	2·73	3·68	4·16	4·75	4·09	5·46	5·70	..
One-half	Mgms.	6·72	9·35	10·21	11·1	14·6	16·79	18·55	20·00
	μl	4·75	8·07	9·73	13·06	14·60	16·39	17·45	18·17
Full	Mgms.	8·90	13·28	14·70	16·08	23·21	30·38	36·51	37·96
	μl	6·05	10·86	14·60	21·91	26·29	29·68	33·71	34·19

It will be seen from the graphs and tables that the digestion of milk can be followed in the dilatometer not only with greater facility and ease but also with greater accuracy. The correlation graphs Figs. 5 and 6, show
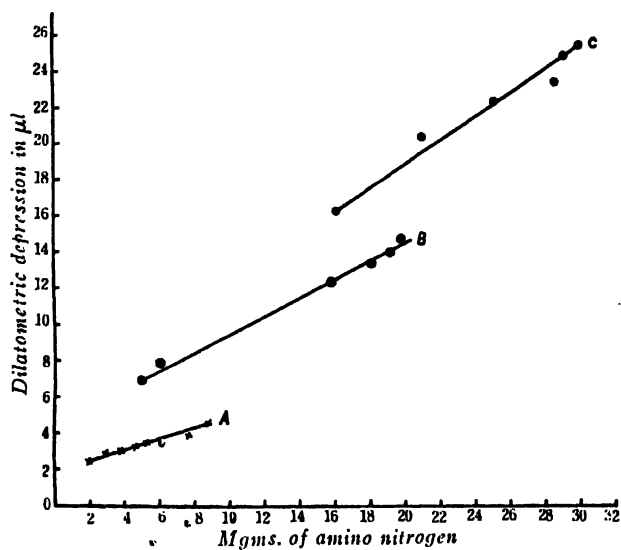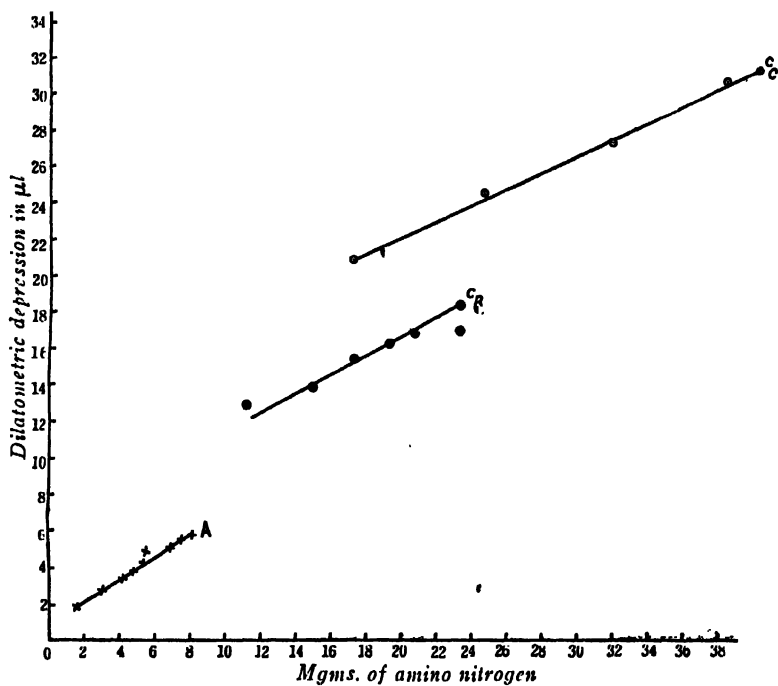
FIG. 5. Correlation Graph for Milk Digestions.

FIG. 6. Casein Digestions—Correlation Graph.

that a strict proportionality exists between the values obtained by the chemical and physical methods, after about 20-60 minutes depending upon the concentration of the colloidal substrate. In the initial stages of the reaction, the disaggregation of the casein particle which accompanies the digestion is accompanied by measurable changes in volume and this circumstance causes the discrepancy observed in the early stages of the reaction.

A study of the results relating to the dilatometer, will show that casein appears to be relatively more digestible than milk, whose absolute protein content capable of undergoing digestion, should be taken as only 87 per cent. of its total nitrogen. For strict comparison, therefore, the values for the casein series have to be multiplied by 0·87, after which the agreement between

TABLE III.

Substrate Concentration		Time in Minutes							
		10	20	30	60	90	120	150	180
One-sixth	Milk	2·37	3·21	3·56	3·92	4·27	4·63	..	
	Casein	2·36	3·20	3·61	4·13	4·34	4·75	..	
One-half	Milk	4·39	7·00	8·76	11·4	12·47	13·53	14·24	14·95
	Casein	4·13	7·02	8·48	11·36	12·70	14·60	16·90	17·4
Full	Milk	4·75	8·07	10·80	16·50	20·53	22·68	23·86	25·17
	Casein	5·26	9·44	12·70	19·06	22·59	25·82	29·32	29·74

the two sets of values becomes very striking particularly for one-sixth and one-half concentrations.

Summary.

1. It has been shown that "in vitro" digestions of milk can be dilatometrically followed not only with greater ease and accuracy but also with considerable economy of research material. A strict proportionality between the dilatometric depression and the release of amino nitrogen has been shown to exist for three different concentrations of milk and casein.

2. The behaviour of the casein particle in cow's milk towards tryptic digestion does not appear to be different from that of the casein particle in artificial solution. A dilatometric study of the rate of digestion of milks from other sources and of those subjected to various chemical and physical treatments, is now in progress.

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INVESTIGATIONS ON METALLIC CONTAMINATION OF FOODS.

Part II. Effect of Cooking and Storage of Foodstuffs in Aluminium Vessels.

BY N. C. DATTA, M.Sc.,*

(*From the Department of Biochemistry, Indian Institute of Science, Bangalore.*)

ALUMINIUM is widely distributed in both animal and vegetable tissues. Myers and Mull (1928) found aluminium varying from 0·2 to 4·5 parts per million in human autopsy tissues. Underhill, Peterman and co-workers (1929) have reported 1·7 to 11·7 p.p.m. of aluminium in human livers and 1·3 to 8·7 p.p.m. in kidneys. Dutoit and Zbinder (1930) reported that aluminium accumulates in lungs, kidney and heart while only traces occur in pancreas. Though the metal is present in varying quantities in different tissues, its exact rôle in human nutrition and metabolism is still obscure.

During recent years, aluminium has come increasingly into prominence and is being largely used for the construction of utensils for household use and industrial food-equipment. It has some advantage over other metals because of its cheapness, light weight and high heat conductivity. Its effect on public health is of considerable interest. Much useful work has already been done in Europe and America to determine whether the quantities of this metal taken along with food are, in any way, injurious to health. Glaister and Allison (1913), Lancet Laboratory Report (1913), Tinkler and Masters (1924), Haase (1926), Serger (1927), Lehmann (1929), Bidault and Blaignan (1930), Fellenberg (1931) and several others have studied the effect of various foods on aluminium and have concluded that the amount of aluminium dissolved under test conditions is too small to have any ill-effect on health. Beal and co-workers (1932) made a complete series of analyses of foodstuffs cooked in aluminium as compared with those prepared in pyrex glass. Those authors showed that in a well-balanced ration about 12 mg. of aluminium would be ingested per day if all the foods were prepared in aluminium vessels, of which, about 5 mg. would be derived from the utensils. These conclusions may not, however, apply to Indian foodstuffs which contain considerable quantities of salts and which, in many cases, are highly

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* Lady Tata Memorial scholar.

acidic in character. It may also be mentioned that in India, the food materials are often stored in the containers for fairly long periods, so that the average daily intake of aluminium will be much higher than that in Europe or America. The present investigation was undertaken to obtain an estimate of the actual quantity of aluminium ingested daily along with food prepared and stored in aluminium vessels and its possible effect on animal growth and metabolism.

Experimental.

A gravimetric method similar to that of Bertrand and Levy (1931) was adopted for the estimation of aluminium which was precipitated and weighed as phosphate. When the amount was small, the precipitate was weighed in a micro-balance. 100 grams of material were carefully ashed in an electrically heated muffle, the temperature gradually raised to a dull red heat. The ash was heated with dilute hydrochloric acid and evaporated to dryness to dehydrate silica. After removing the silica, the solution was treated with ammonium phosphate and ammonium chloride, cooled, and made faintly alkaline with ammonia to precipitate the phosphates of iron, aluminium and calcium. The solution containing the phosphate still at about $20°$ C. was acidified with concentrated acetic acid constantly stirred to dissolve the calcium phosphate, the pH being adjusted to $4·2$. The iron and aluminium phosphate was collected by centrifuging, dissolved in hot hydrochloric acid and diluted to 300 c.c. The iron was reduced with thiosulphate and the aluminium reprecipitated with ammonium phosphate and ammonium acetate. The solution was boiled for half an hour to expel SO_2, the precipitate allowed to settle and filtered. The precipitate was redissolved and reprecipitated in presence of thiosulphate, filtered and washed with hot water and ignited to constant weight at red heat.

The aluminium content of some of the commoner foodstuffs is given in Table I, which shows that they all contain small quantities of that metal. This will no doubt have to be consumed irrespective of the type of vessels used for cooking and storing them. The problem before us therefore is to find out whether the excess of aluminium added to the food material from the vessels is deleterious to health.

Dissolution of aluminium by various foodstuffs :—Water.—Tap water (100 c.c. ; pH $6·9$) was stored in different aluminium vessels for four days. The vessels were slightly attacked with the formation of minute pits. Although the quantity of aluminium dissolved was only $1·43$ mg. on an average, the appearance of water was changed with suspended aluminium hydroxide, which was formed around those pin points in the form of small beads.

TABLE I.

Aluminium content of some Indian foodstuffs.

Foodstuff	Aluminium as p.p.m. on dry basis
Rice	17·70
Whole Wheat ·	4·5
Wheat Flour (Bazaar Sample)	38·7
Pigeon Pea	9·86
Green Gram	7·5
Potato (pealed)	8·7
Peas	9·2

Milk and milk products.—The amount of aluminium dissolved on boiling milk in an aluminium vessel is insignificant. Acid curd dissolves only a small quantity of aluminium. 150 c.c. of curd prepared and stored for 21 hours dissolved only 19·6 mg. of that metal in 15 days. Aluminium vessels appear to be well suited for milk and milk products.

TABLE II.

Total acidity of curds prepared in glass and aluminium vessels.

Substance	Titratable acidity as c.c. of normal acid	
	Glass vessel	Aluminium vessel
100 c.c. of whey from acid curd Sample I ..	13·74	13·10
Sample II ..	11·62	9·51
Sample III ..	12·66	11·46
Sample IV ..	13·74	12·26

The course of change in total acidity of curd stored in glass and aluminium vessel was studied. The acidity was determined in the clear whey obtained by centrifuging the curd and filtering the slightly turbid supernatant liquid.

It was observed that at a given temperature and in a given time, the development of total acidity of curd stored in aluminium vessel was slightly lower than that stored in glass vessel.

TABLE III.

Aluminium content of foodstuffs stored in aluminium vessels.

Foodstuffs	pH	Duration of storage (in hrs.)	Aluminium p.p.m.	Remarks
Vinegar	..	40	2.60	10 c.c. diluted to 100 c.c.
Orange juice	3.5	24	6.21	Stored as such.
Lime juice	2.2	48	7.54	10 c.c. diluted to 100 c.c.
Water extract of green mango	3.0	40	9.76*	Stored as such.
Do. ..	..	48	11.76	do.
Tamarind water	2.8	24	18.58	do.
Tamarind water with 1 per cent. salt	3.0	24	28.18	do.
Tamarind water with 2 per cent. salt	..	24	39.8	do.
Rasam* (cold)	4.1	18	14.86	do.
Butter-milk	3.8	24	10.65	do.
Vegetable salad with Vinegar and salt	..	5	19.74	do.
Lime pickles (semi-solid) ..	..	20 days	1153	do.

* A South Indian preparation of soup containing cooked pigeon pea (or its extract), tamarind, salt and spices.

Acid foods dissolved a small quantity of aluminium from the vessels during prolonged storage. With tamarind the quantity of aluminium dissolved was higher than with other fruit juices : its corrosive action was increased by the presence of salt. Since various preparations containing tamarind solution and salt are concentrated and stored in such vessels, a systematic investigation on the effect of these on aluminium was carried out.

Effect of salt and tamarind solution on aluminium.—Salt solution was found to attack aluminium vessels during storage, the rate of corrosion being very nearly proportional to the time of exposure. The product of reaction was found to be aluminium hydroxide which formed a layer along the entire surface of the vessels exposed to liquid.

TABLE IV.

Aluminium content of foodstuffs after cooking and storage.

Substance	Duration of cooking (in min.)	Aluminium content after cooking (in mg.)	Duration of storage (in hrs.)	Aluminium content after cooking and storing (in mg.)
2.5 per cent. sodium chloride solution 200 c.c. ..	40	0.77	20	5.73
Tamarind water 200 c.c. (pH 3.0)	40	1.70	40	5.30
Tamarind water with 1 per cent. salt 200 c.c. ..	30	2.77	40	14.64
Rasam (Liquid) 100 c.c. ..	30	1.83	24	11.07
Tomato juice with 1 per cent. salt 100 c.c. ..	30	2.10	..	..
Tomato juice, 100 c.c. with 1 per cent. salt ..	60	concentrated to a thick syrup	20	14.4
Red grape juice 100 c.c. ..	60	do.	20	3.97

The quantity of aluminium in the food immediately after cooking was comparatively small. On prolonged storage, however, the quantities in solution increase considerably. Even then, if all the food cooked and stored in aluminium vessel as mentioned above are included in the diet of an individual, the total quantity of aluminium thus taken will be only 50 mg. daily.
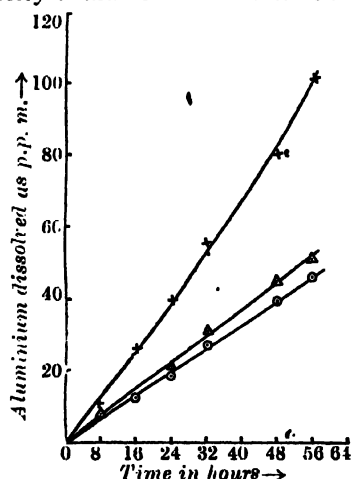

FIG. 1.   Dissolution of aluminium by tamarind and salt solution.
    x—x Tamarind water pH 2·8.
    ▲—▲ 2% salt solution.
    ⊙—⊙ Tamarind water and 2% salt.

The rate of solution of aluminium with tamarind solution (pH 2·8) was slightly lower than that with 2 per cent. salt solution (Fig. 1) but when a solution of tamarind with 2 per cent. salt was stored for several hours in an aluminium vessel, it was found that the amount of aluminium in solution at any period was very nearly equal to the sum total of the quantities dissolved by salt and tamarind solution taken separately so that each seems to act independent of the presence of other. The aluminium hydroxide formed by the action of salt was dissolved by the acid of the tamarind solution. The aluminium dissolved by the combined action of acid and salt was found to be 102 p.p.m. during 56 hours of test period.

TABLE V.

Effect of cooking tamarind water with 2 per cent. salt.

Duration of cooking in mins.	Original Volume in c.c.	Final Volume in c.c.	Aluminium dissolved (in mg.)
35	125	100	2·77
75	125	60	5·55
95	125	40	7·50

Aluminium vessels are attacked on boiling acid solutions containing tartaric acid and salt. The quantity of aluminium dissolved becomes fairly large if the boiling is continued for a long time. The corrosion starts at the air-liquid junction with the formation of a white stain. The hot solutions after boiling should not be stored in the same vessel where corrosion has already started, for if stored for a long time, pin holes appear around the points of previous attack.

The effect of pH *and titratable acidity on the solubility of aluminium.*— Tamarind solution (100 c.c., pH 2·8) and diluted lime juice (100 c.c., pH 2·2) were taken and to these, varying quantities of tartaric and citric acid respectively were added. The solutions were stored in aluminium vessels for 48 hours and the amount of aluminium dissolved estimated. The titratable acidity and the pH value of the liquids had no appreciable effect on the solubility of aluminium under the test conditions. Tartaric acid solution dissolved about nine times the amount of aluminium than citric acid solution having nearly the same pH and titratable acidity.

Feeding experiments.—The results of animal experiments carried out in other countries have shown that aluminium compounds given by mouth produce no harmful effect on the system. McCollum, Rask and Becker

TABLE VI.

Substance	pH	Titratable acidity as c.c. of normal acid	Aluminium dissolved as p.p.m.
100 c.c. of tamarind solution	2·8	2·53	39·43
100 c.c. of tamarind with 1 g. of tartaric acid	2·4	13·63	58·13
100 c.c. of tamarind with 1·5 g. of tartaric acid	2·2	19·02	62·13
100 c.c. of diluted lime juice (35 c.c. to 100 c.c.)	2·2	20·6	7·32
100 c.c. of lime juice with 2·5 g. of citric acid	2·2	31·2	7·98

(1928) observed that when aluminium chloride is added to the diet of growing rats to the extent of 0·7 per cent. of aluminium of the diet, there was no noticeable deleterious action on growth and general well-being of the animals as judged by external appearance and autopsy. Similar observation has also been made by Myers and Mull (1928) who carried out investigation covering four generations of rats, giving them 2 mg. of aluminium in the form of potassium aluminium sulphate. They observed that aluminium-fed animals had slightly greater initial growth than the control. Mackenzie (1932) added aluminium as phosphate to the diet of rats but observed no noticeable difference between control and aluminized rats as regards growth and fertility. Mackenzie also (1931) observed that rats receiving diet containing aluminium excrete the metal entirely by way of alimentary tract. There is no clear evidence that any of the aluminium is excreted in the urine. This would suggest that there is no absorption by the internal organs.

In all the experiments referred to above, aluminium was added in the form of chloride, sulphate or as phosphate to the food material which relates more or less to neutral substances. It is probable however that in acid solutions aluminium might exert some harmful effect. Experiments were therefore carried out by feeding animals with acid foodstuffs prepared and stored in aluminium vessels. Since there is belief among a certain section of people in India that curds prepared in aluminium vessels are injurious to health, the effect of feeding curd stored in aluminium vessels were tried on the animals.

Young rats at an age of 30–35 days were kept on a diet of wheat chappati supplemented with 20 c.c. of curd stored in aluminium vessel for 24 to 30 hours. A set of rats were also kept with curd prepared in glass vessel for control. The feeding experiment was conducted for six months.

In Fig. 2, is represented the average growth curve of rats getting curd prepared and stored in both glass and aluminium vessels. It may be observed from Fig. 2 that there is no difference in growth rate between the two sets of rats.
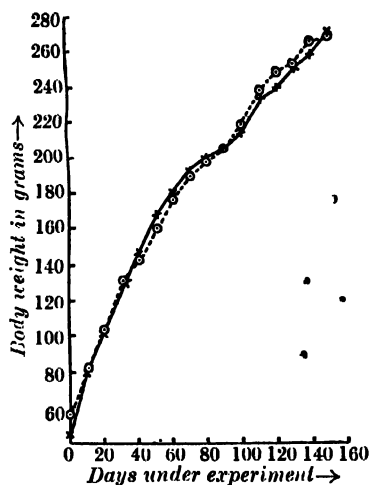

FIG. 2. Growth rate of rats on milk curd stored in aluminium and glass vessels.

⊙··⊙ Curd stored in glass vessel.
✕—✕ Curd stored in aluminium vessel.

TABLE VII.

Average increase in weight of a group of rats maintained on wheat chappati and curd prepared in glass and aluminium vessels.

Food prepared in	Number of rats	Sex	Average body weight in grams		
			Original	Final	Increase
Glass	4	♂	50	282	232
	5	♀	46	170	124
Aluminium ..	6	♂	44	283	239
	4	♀	43	186	143

The results of Table VII will show that the growth rates of rats fed with curd prepared in aluminium vessel were as good as those of the controls. The animals maintained perfect health throughout the period of observation.

Effect on reproduction.—The male and female rats of the previous experiment which were kept in separate cages before were mated together, and the same diet was continued. The females of both the control group and those getting curd from aluminium vessel gave birth to the same number of litters (11 on an average) out of which eight survived in each case.

In the second series of experiment rats were kept on rice, rasam and vegetables (without milk and milk products) which therefore represents poor specimen of diet, prepared and stored in aluminium vessels. The growth rate of both the group of rats were almost the same, although the control animal had slight increased growth towards the end, the difference was not, however, very significant (Fig. 3).
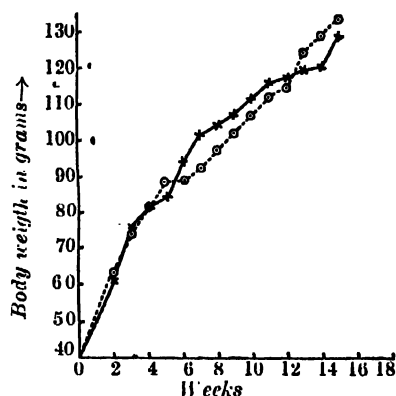

FIG. 3.   Average growth rate of female rats maintained on rice, rasam and vegetables prepared in glass and aluminium vessels.

o--o   Food prepared in glass vessel.
x--x   Food prepared in aluminium vessel.

Discussion.

The results of the present enquiry show that aluminium vessels are only slightly attacked by fruit and vegetable juices when stored at ordinary temperature. With tamarind solution (containing tartaric acid) the quantity of aluminium in solution was higher than with other fruit juices. Mrak and Cruses (1929) observed that aluminium is more resistant to pure citric and malic acids than to tartaric acid. Colobraro (1931) boiled 100 c.c. of solutions of tartaric and citric acid in aluminium vessel for one hour. In 100 g. of the substance the following value of aluminium was found. 0·25 per cent. of tartaric acid dissolved 6·11 mg. whereas 3 per cent. citric acid

dissolved 6·47 mg. Result of Table VI shows that tartaric acid solution pH 2·2 and containing 19·02 c.c. of normal acid dissolved much more aluminium than citric acid solution having pII 2·2 and containing acid equivalent to 20·6 c.c. of normal acid. It is possible therefore that the amount dissolved by acid foodstuffs depends on the nature of organic acid and the buffering capacity of the food material.

Although salt solutions attack aluminium vessel during boiling and storage, yet acid foodstuffs containing salt dissolve only a small amount of aluminium in the ordinary process of cooking. If all the articles of food containing salt are prepared in aluminium vessel in the usual way and stored for reasonable length of time, the amount of aluminium that will be added to the diet of an individual under Indian conditions will be less than 50 mg. per day.

The quantity of aluminium dissolved by foodstuffs containing salt increases if boiling is continued for a long time. Corrosion starts at the air-liquid junction. If the solution after boiling is left in those containers for considerable length of time, pits are formed around those white stains. Pin pricks have also been observed in many aluminium vessels of household use. The formation of such pits in aluminium presents a very undesirable appearance and also effects durability. The extent of such corrosion may depend on the concentration of salt, time of exposure, the quality of metal or alloy employed in the construction of vessels.

The mechanism of action of salt on aluminium with reference to dissolution of metal and pit formation using various samples of aluminium and with different concentration of salt both in presence and absence of air and also method for protecting such type of corrosion requires further elucidation and will form the subject of further investigations.

Summary.

1. Aluminium vessels appear to be well suited for milk and milk products.

2. Fruit and vegetable juices dissolve only a small amount of aluminium from utensils during storage. The amount of aluminium dissolved is not a function of titratable acidity but possibly depends on the nature of organic acid present and also the buffering capacity of food material.

3. The corrosive action of acid foods on aluminium is increased by the presence of salt. The amount of aluminium dissolved by tamarind solution containing salt during storage is very nearly equal to the sum total of the amount of aluminium dissolved by acid and salt taken separately, so that each seems to act independent of the presence of the other.

4. The amount of aluminium dissolved in the ordinary process of cooking is very small, but in cases when acidic foodstuffs containing salt are cooked and stored for fairly long periods in aluminium vessels, the maximum that may be added to the daily diet from utensils will be about 50 mg.

5. Acidic foodstuffs containing salt after boiling in aluminium vessels should not be left long in the same vessel where corrosion has already started since in many cases, pin pricks may appear at the sides around the points of previous attack.

6. Feeding experiments with rats have shown that food prepared in aluminium vessels has no harmful effect on growth, reproduction and general well-being of the animals.

My thanks are due to Prof. V. Subrahmanyan for his kind help and advice in the course of the investigation.

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EXPLANATION OF PLATE.

Fig. 4.—Showing the scratches on an unused vessel due to polishing.

Fig. 5.—Showing nature of corrosion on storing tamarind water and salt for 48 hours.

Fig. 6.—Showing the complete and uniform etching away of the surface on storing lime pickles for 20 days.

Fig. 7.—Enundations with stains formed at the side of the vessel near air-liquid junction on boiling acid foods containing salt.
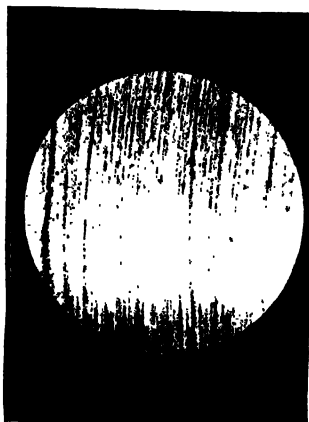
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FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

DILATOMETRIC STUDIES IN THE ENZYMIC HYDROLYSIS OF POLYSACCHARIDES.

III. Hydrolysis of Starch, Amylose and Amylopectin by Takadiastase.

BY H. B. SREERANGACHAR.

(*From the Department of Biochemistry, Indian Institute of Science, Bangalore.*)

IT has been shown in our previous communications (Sreenivasaya, Sreerangachar and Keshava Iyengar, 1934 ; Sreenivasaya, Sastri and Sreerangachar, 1934 ; Sreerangachar and Sreenivasaya, 1934) that in the case of colloidal substances whose molecular weights are unknown, the contraction constants could be more appropriately expressed on the basis of release of one of the products of their hydrolysis. Thus, during the hydrolysis of starch the dilatometric depression per millimol release of maltose gives the constant of the system. The course of hydrolysis can also be followed by a determination of optical rotation and the fall in rotation correlated with the dilatometric depression.

The initial stages of the enzymic digestion of colloids and particularly those of the hydrophylic nature, are complicated by changes taking place in the physical condition of the substrates prior to cleavage. The destruction of their colloidality causes a disturbance in the water balance of the system and it has been observed both in the case of proteins (Sreenivasaya, Sastri and Sreerangachar, *loc. cit.*) and starch (Sreerangachar and Sreenivasaya, *loc. cit.*) that during this period the dilatometric depression is not strictly proportional either to the liberation of the products of hydrolysis or the fall in rotation. After this period, however, they bear a linear relationship to each other. Thus for malt-diastase-starch system the depression per millimol release of maltose is $3 \cdot 9$ while the depression per degree fall in rotation is $6 \cdot 8$.

The present investigation relates to a dilatometric study of the hydrolysis of starch and its two main constituents amylose and amylopectin by Takadiastase which is classed by Kuhn (Richard Kuhn, 1924) as an α-diastase.

Experimental.

Lintner's soluble starch and a preparation of Takadiastase (Parke Davis & Co.) were used for the first series of experiments described in this communication. Potato starch was a preparation from Kahlbaum. Amylopectin

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was prepared from potato starch by the method of Ling and Nanji (Ling and Nanji, 1923), a 2% starch paste being frozen to −10° C. with ice and salt mixture and kept at that temperature for 10-12 hours. On slowly raising the temperature of the frozen mass to 60° C. accompanied by constant stirring, the amylose goes into solution leaving a centrifugable precipitate of amylopectin which is purified by washing on the centrifuge.

The amylopectin (A_1) thus obtained can be precipitated with alcohol and dried into the form of a white powder, which, however, was found to be difficultly soluble in water. In an experiment the wet precipitate was therefore directly dispersed in hot water which on boiling gelatinised to a thick viscous paste. This was made up to the required strength with the phosphate buffer at 5·3.

Another preparation of amylopectin (A_2) was obtained by the method of Eckert and Marzin (Eckert and Marzin, 1932). Potato starch (25 g.) was refluxed with 300 c.c. of methyl alcohol and 2 c.c. of concentrated sulphuric acid over a water bath until the starch did not yield any blue colouration with iodine. The granular white suspension consisting of amylopectin is filtered off on a gooch, washed repeatedly with alcohol to remove the last traces of acid, finally with ether and dried in a desiccator.

In the case of soluble starch two concentrations of substrate (1 and 2 per cents.) have been employed while in the case of other preparations only one concentration has been employed. A one per cent. concentration of the enzyme was employed in all cases, and the volume ratio of substrate to enzyme was 10 : 1. All experiments were carried out at 30° C. and p_H 5·3 maintained by Sorenson's phosphate buffer. The dilatometric determinations were carried out in the two bulb dilatometer described before (1932). The reaction was simultaneously carried out in a separate flask from which 20 c.c. aliquots of the reaction mixture were drawn at known intervals and treated with 5 c.c. of alkali solution to arrest the enzyme action and muta rotation. This mixture was subsequently employed for the determination of the reducing sugars by Bertrand's method and of optical activity. In the case of potato starch and amylopectin (A_1) measurements of optical rotation could not be carried out on account of the high opalescence of the reaction mixture.

The determination of the optical rotation was carried out in a 100 mm. tube at 30° C. It was observed that the use of N/2 sodium hydroxide to arrest the enzyme action led to the destruction of some sugars thus giving a higher value for the fall in rotation. But when however a solution of 2N sodium carbonate is used for the purpose, the rotations observed are constant
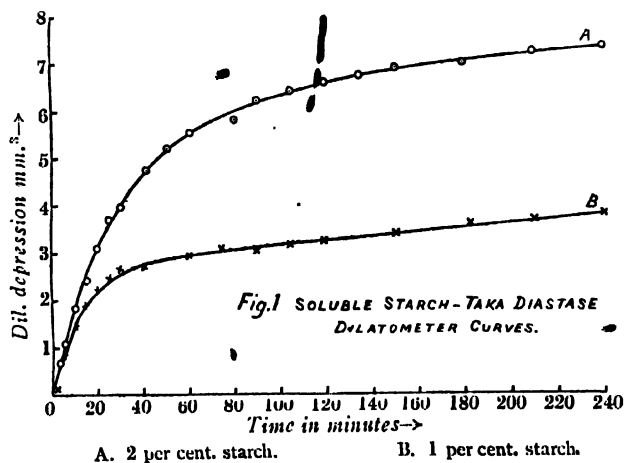
for a considerable length of time showing thereby that there is no destruction of sugars within this period as revealed by Table I.

TABLE I.

Time allowed for Reaction	Duration of Contact of the Reaction Mixture	Concentration of the Alkali added	
		N/2 NaOH	2N Soda
30 mins.	15 mins.	2·38	2·52
	180 ,,	2·16	2·54
	20 hrs.	1·93	2·56
	43 ,,	1·58	2·53
120 mins.	15 mins.	2·23	2·39
	180 ,,	—	2·37
	20 hrs.	—	2·35
	43 ,,	1·42	2·36

For convenience the results are given in two sets of graphs, one dealing with the hydrolysis of soluble starch and the other with potato starch and amylopectin.

Figs. 1, 2 and 3 give the experimental values for dilatometric depressions, maltose values and changes in optical rotation when soluble starch is hydrolysed by Takadiastase. The depression in mm.³ per millimol release of maltose for different intervals of time, calculated from these graphs, is given in Table II, while Table III gives the depression in mm.³ per degree change of rotation. Fig. 4 shows the relationship between the dilatometric depressions on the one hand and the corresponding maltose values and optical rotations on the other.
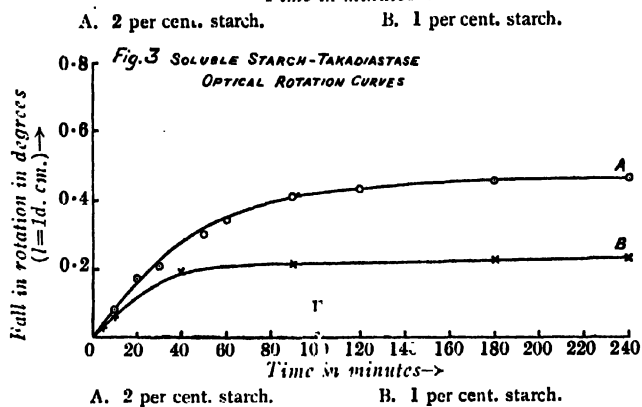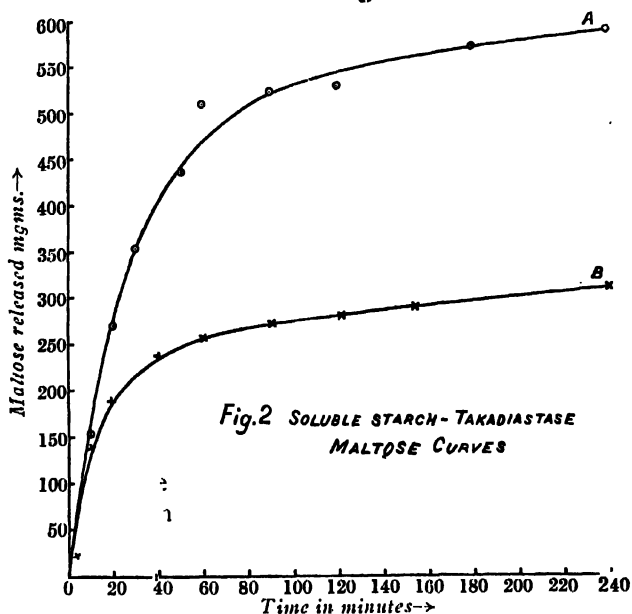

Fig. 1 SOLUBLE STARCH–TAKA DIASTASE DILATOMETER CURVES.

A. 2 per cent. starch.   B. 1 per cent. starch.

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Fig.2 SOLUBLE STARCH - TAKADIASTASE
MALTOSE CURVES

Time in minutes→

A. 2 per cent. starch.   B. 1 per cent. starch.

Fig.3 SOLUBLE STARCH-TAKADIASTASE
OPTICAL ROTATION CURVES

Time in minutes→

A. 2 per cent. starch.   B. 1 per cent. starch.

TABLE II.   Soluble Starch.

Dilatometric Depression in $mm.^3$ per millimol release of maltose.

Substrate Concentration per cent.	Time in Minutes						
	30	40	60	90	120	180	240
1·0	4·0	4·0	4·0	4·1	4·1	4·1	4·2
2·0	4·1	4·1	4·0	4·0	4·0	4·0	4·1

Fig.4 Soluble starch Correlation graphs
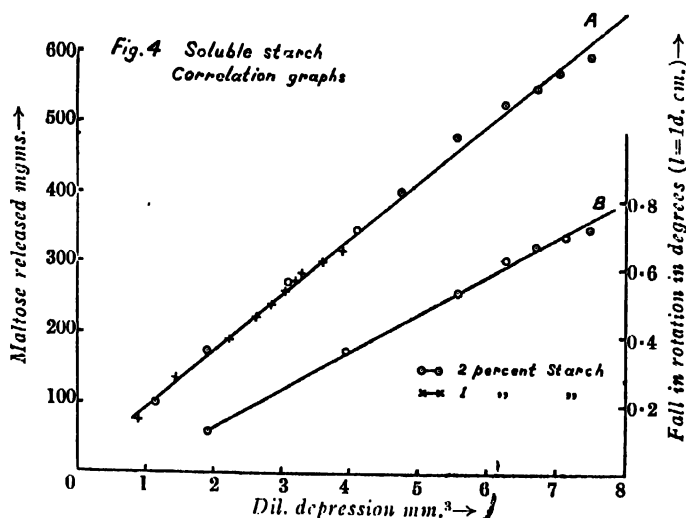
A. Correlation between dil. depression and maltose released.
B. Correlation between dil. depression and fall in optical rotation.

TABLE III.

Dilatometric Depression in mm.³ per degree change of rotation.

Substrate Concentration per cent.	Time in Minutes					
	30	60	90	120	180	240
1·0	10·5	9·8	10·0	10·0	10·6	10·1
2·0	..	10·7	10·2	10·3	10·5	10·7

An examination of these graphs and tables reveals that there is a linear relationship between the dilatometric fall and the amount of reducing sugars released during the hydrolysis. Such a relationship has also been found to exist between the dilatometric and the polarimetric values as can be observed from Fig. 4. The Kinetics of the reaction can therefore be followed by any of the three methods, all of them being interrelated to each other as already shown. The average dilatometric depression per millimol release of maltose is 4·0 mm.³ while per degree fall of rotation the average dilatometric value amounts to 10·3 mm.³

Hydrolysis of Potato Starch and Amylopectin.

In order to investigate individually the volume changes involved during the hydrolysis of the two main constituents of starch, amylose and

amylopectin, another substrate, namely, potato starch in which these two constituents occur in a different proportion, was also subjected to the enzymic digestion. Later on amylose and amylopectin were obtained separately by methods already mentioned and employed as substrates. Of the amylopectins, only the amylopectin (A_2) prepared by methyl alcoholic HCl method admitted of a determination of the optical rotation while both potato starch and the other amylopectin preparation (A_1) were too viscous and turbid for the purpose.
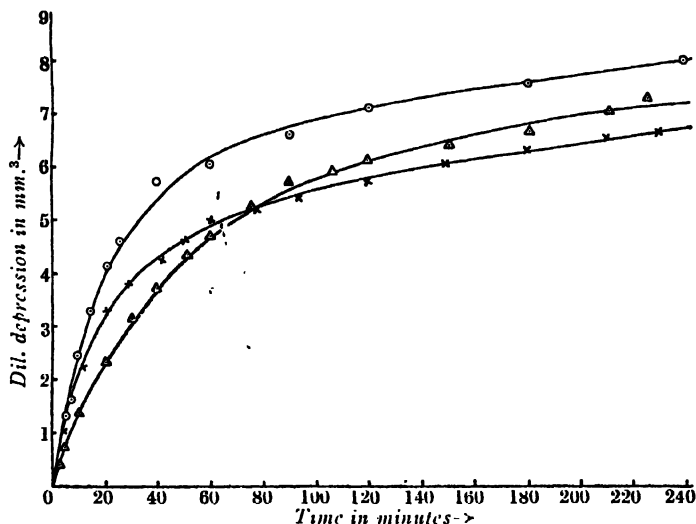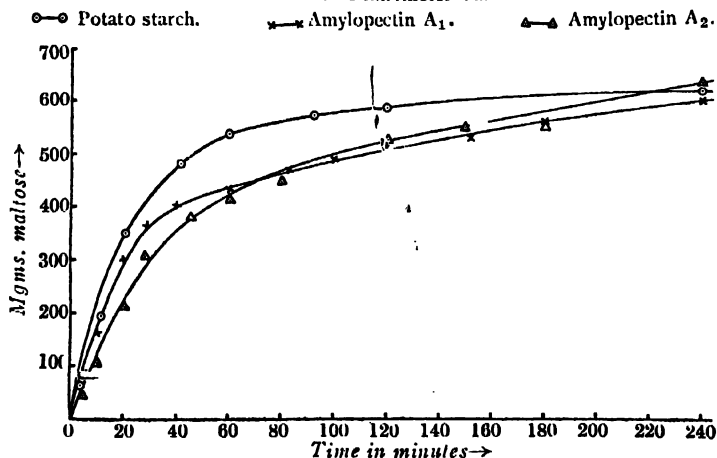

FIG. 5. Dilatometer Values.

⊙—⊙ Potato starch.   ✕—✕ Amylopectin A_1.   ▲—▲ Amylopectin A_2.

FIG. 6. Maltose Values.

●—● Potato starch.   ✕—✕ Amylopectin A_1.   ▲—▲ Amylopectin A_2.

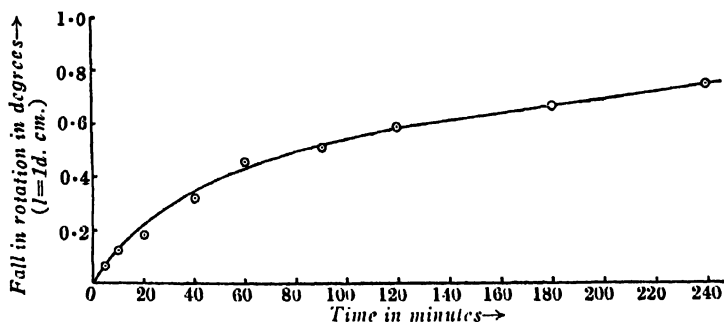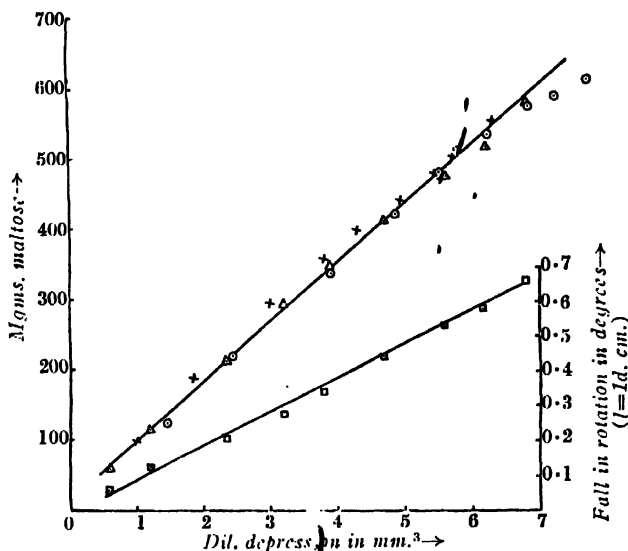FIG. 7. Optical Rotation Values for Amylopectin A₂.

FIG. 8. Correlation Graphs.

O—O Potato starch dilatometer / maltose
x—x Amylopectin ,,   ,,   ,,
△—△ ,,   ,,   A₂ ,,   ,,
□—□ ,,   ,,   ,, dilatometer / rotations.

Figs. 5 and 6 respectively represent the experimental results of dilatometric depressions and maltose values while changes of optical rotation for the amylopectin (A₂) are given in Fig. 7. The correlations between the dilatometric depressions and the maltose values on the one hand and the fall in the rotation on the other are given in Table IV and graphically represented in Fig. 8.

Table V gives the summary of all the results obtained during the investigation.

TABLE IV.

Substrate	Concentration of Substrate per cent.	Time in Minutes.						
		30	40	60	90	120	180	240
Depression per millimol release of maltose.								
Potato starch	2	3·9	4·0	4·0	4·0	4·0	4·1	4·2
Amylose	—	—	—	—	3·6	3·7	3·6	—
Amylopectin A$_1$	—	3·6	3·7	3·8	3·8	3·8	3·8	—
Amylopectin A$_2$	2	3·5	3·8	3·9	4·0	4·0	4·0	—
Depression per degree fall in rotation.								
Amylopectin A$_2$	2	11·5	10·6	10·7	10·6	10·6	10·5	10·3

TABLE V.

	Depression per millimol release of maltose	Depression per degree fall in rotation
Soluble starch	4·0	10·3
Amylose	3·6	—
Amylopectin A$_1$	3·6	—
Amylopectin A$_2$	3·7	10·7
Potato starch	4·0	—

It is shown by Table V that the values for the dilatometric depression per millimol release of maltose for these several substrates, namely, soluble starch, potato starch, amylose and amylopectin, all lie very near each other and in the proximity of the value obtained for soluble starch-malt diastase system. The two amylopectin preparations which differed considerably in their viscosities due probably to the dephosphorolysing of A$_2$ during its preparation, agree very strikingly with regard to their constants. Further, the dilatometric depressions per degree fall in rotation in the case of soluble starch and amylopectin A$_2$ closely agree with each other. These

results point to the conclusion that the depression in volume registered by the dilatometer is related to the release of one mol of maltose and appears to be independent of the nature of the substrates, amylose and amylopectin.

These studies also indicate the possibility of employing the dilatometer to determine the relative digestibilities of different starches on the basis of the maltose released.

In conclusion, I wish to express my grateful thanks to Mr. M. Sreenivasaya for his kind help rendered throughout this work.
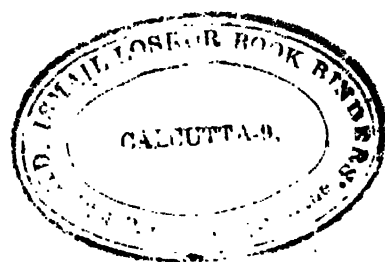
Summary.

(1) A dilatometric study of the hydrolysis of soluble starch with taka-diastase has been carried out in the two-bulb dilatometer.

(2) The depression per millimol release of maltose and the depression per degree fall in rotation are found to be 4·0 and 10·7 respectively.

(3) Potato starch and the amylopectins prepared by two distinctly different methods have also been subjected to similar dilatometric studies. The depressions per millimol release of maltose are 4·0, 3·6 and 3·7 respectively. In the case of amylopectin prepared by Eckert and Marzin's method, the depression per degree fall in rotation is 10·7, a value which agrees very well with that for soluble starch.

(4) It is suggested that the dilatometer offers a convenient method for studying the relative digestibilities of starches from various sources.

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